

1 **An Empiricist's Guide to Modern Coexistence Theory for Competitive Communities**

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## 14 **Abstract**

15 While most ecological theories have historically invoked niche differences as the primary  
16 mechanism allowing species coexistence, we now know that species coexistence in competitive  
17 communities actually depends on the balance of two opposing forces: niche differences (ND)  
18 that determine how species limit their own growth rate versus that of their competitor, and  
19 relative fitness differences (RFD) that establish competitive hierarchies among species. Several  
20 different empirical methods have been proposed for measuring ND and RFD in order to make  
21 predictions about coexistence of species, yet it remains unclear which method(s) are appropriate  
22 for a given empirical study and whether or not those methods actually yield the same  
23 information. Here we summarize and compare five different empirical methods, with the aim of  
24 providing a practical guide for empiricists who want to predict coexistence among species. These  
25 include two phenomenological methods that estimate ND and RFD based on observing  
26 competitive interactions among species; two mechanistic methods that estimate ND and RFD  
27 based solely on information about species' resource requirements; and a fifth method that does  
28 not yield ND and RFD but describes the impacts of those forces within communities. Based on  
29 the specific requirements, limitations, and assumptions of each approach, we offer a series of  
30 decision steps that can be used to determine which method(s) are best for a given study system.  
31 In particular, we show there are important tradeoffs between mechanistic methods, which require  
32 detailed understanding of species niches and physiology but are more tractable experimentally,  
33 and phenomenological methods which do not require this detailed information but can be  
34 impractical for some study designs. Importantly, we show that although each method can be used  
35 to estimate ND and RFD, the methods do not always yield the same values. Therefore we caution  
36 against future syntheses that compile these estimates from different empirical studies. Finally, we

37 highlight several areas where modern coexistence theory could benefit from additional empirical

38 work.

39

40

## 41 **Introduction**

42           Throughout most of the history of community ecology, it has been assumed that niche  
43 differentiation among species is primary biological mechanism that can offset the negative  
44 impacts of interspecific competition on species coexistence (Volterra 1931, Gause 1934, Tilman  
45 1982, Leibold 1995, Chase and Leibold 2003). This idea originated when Volterra (Volterra  
46 1931) introduced a dynamic model of competition that became the foundation for the  
47 competitive exclusion principle, which states that if two species have identical niche  
48 requirements then one of them will inevitably become locally extinct (Gause 1934). The  
49 competitive exclusion principle led to two conclusions about coexistence in competitive  
50 communities: 1) species will coexist only if they are limited by different resources (or  
51 consumers) at the same location and time, or if they partition resources (or consumers) in space  
52 or time and, as a result, 2) ecosystems should contain only as many species as there are limiting  
53 resources (or consumers) (Rescigno and Richardson 1965, MacArthur and Levins 1967,  
54 MacArthur 1970, Tilman 1977, Leibold 1995). Nearly all subsequent hypotheses to explain  
55 coexistence have argued that biodiversity exists because of such niche differences among  
56 species.

57           While niche partitioning remained the theoretical basis for understanding species  
58 coexistence, empiricists and theorists struggled to reconcile the rich biodiversity that exists in  
59 many of the world's ecosystems with the prediction that competitive communities should contain  
60 only as many species as there are limiting resources (or consumers) (Hutchinson 1961, Oksanen  
61 et al. 1981, Huisman and Weissing 1999). But starting in 2000, theories of species coexistence  
62 began to undergo a major revision. In 2001, Hubbell published The Unified Neutral Theory of  
63 Biodiversity (Hubbell 2001), which argued that patterns of biodiversity in nature can be

64 explained by a simple model that does not invoke niche differences among species. According to  
65 Hubbell's theory, species coexist not because they are different, but because their demographic  
66 parameters are identical, or nearly so, such that the consequences of their interactions are  
67 'neutral' (i.e. essentially equal among all species). Based on this neutral theory, Hubbell argued  
68 that the biodiversity we observe in nature can be explained by a series of stochastic events that  
69 cause some populations to become dominant while others exhibit random walks toward  
70 extinction.

71       Even as Hubbell was developing his neutral theory, Chesson (2000) was completing a  
72 ground-breaking theory of coexistence that would provide a framework for integrating the niche  
73 and neutral perspectives on biodiversity. Chesson's coexistence framework was built on his  
74 insight into the invasibility criterion: a pair of species can coexist only if each species is capable  
75 of invading a steady-state population of its competitor. Chesson showed how a species' long-  
76 term growth rate when invading a resident species can be decomposed into two general terms,  
77 which he called stabilizing and equalizing forces. Stabilizing forces cause species to limit their  
78 own growth rate more than they limit the growth rate of other species (intra > interspecific  
79 competition). These stabilizing forces, also known as niche differences (ND), occur when  
80 species partition limiting resources in space or time, or when they experience differential  
81 consumption by consumers. In contrast, equalizing forces minimize differences in competitive  
82 abilities among species. Equalizing forces, which have also been called relative fitness  
83 differences (RFD), are the result of inherent variation in biological traits such as minimum  
84 requirements for shared resources or consumers, differential resistance to consumers, or  
85 differences in potential growth rates (Adler et al. 2007, Levine and HilleRisLambers 2009,  
86 HilleRisLambers et al. 2012).

87 Chesson showed it is the balance of these two forces – RFDs that establish competitive  
88 hierarchies, and NDs that prevent competitive exclusion – that ultimately determines whether  
89 species maintain non-negative long-term growth rates in competitive communities (Chesson  
90 2000). For a pair of species to coexist, ND must be sufficiently large to offset and stabilize the  
91 competitive hierarchies generated by RFDs. When two species exhibit identical niches (ND  
92 equals 0), their RFD alone determines the competitive hierarchy and which species will become  
93 extinct. It has subsequently been shown that Hubbell's neutral theory represents a specific,  
94 limiting case of Chesson's coexistence theory where NDs and RFDs are both zero, causing the  
95 outcome of competition to be approximated by a random walk toward extinction (Adler et al.  
96 2007). Stabilizing and equalizing forces have been identified in both fluctuation-dependent  
97 mechanisms (e.g. storage effects) and fluctuation independent mechanisms of coexistence (e.g.  
98 competition for a limiting resource) (Miller and Klausmeier 2017, Barabas et al. 2018). Thus,  
99 Chesson's inequality provides a general framework for predicting species coexistence.

100 Since the development of Chesson's theory, much attention in ecology has turned  
101 towards the empirical estimation of ND and RFD in order to determine how these two forces  
102 contribute to coexistence in real communities. As a growing number of empiricists have tried to  
103 quantify ND and RFD in their individual study system, the number of different empirical  
104 approaches proposed for doing so has also grown. However, the various methods for  
105 implementing modern coexistence theory in empirical studies have been derived from different  
106 models of species interactions, make different assumptions, and use different experimental  
107 designs. Therefore, it remains unclear which method(s) are best suited for a given study, whether  
108 the methods give comparable estimates of ND and RFD, and whether the methods actually give  
109 the same prediction regarding coexistence. If Chesson's theory is to become widely implemented

110 in empirical studies and in applied contexts, we need a ‘users guide’ to help ecologists determine  
111 which empirical approach meets their needs.

112 Here we provide a summary and comparison of four methods that have been proposed to  
113 measure ND and RFD empirically, and a fifth method that does not give estimates of ND and  
114 RFD but has been used to predict coexistence based on Chesson’s theory. In Part 1 of our paper,  
115 we describe the theoretical background of each method, illustrate how it can be implemented  
116 empirically, and ask whether the methods yield the same estimates of ND and RFD and make the  
117 same predictions regarding coexistence. In Part 2 of the paper we provide a list of decision steps  
118 to guide empiricists in selecting the most appropriate method(s) for their study system and  
119 contrast the methods in terms of the amount of effort required to implement them. In Part 3, we  
120 discuss the main advantages and disadvantages of the methods and make some suggestions for  
121 future empirical work on coexistence theory.

122

### 123 **Part 1. Summary of Five Empirical Methods for Implementing Chesson’s Theory**

124 In this part of the paper we first review the fundamentals of Chesson’s theory that are  
125 most relevant to empirical work on competition and coexistence. We then summarize each of  
126 five empirical methods that have been used to measure niche differences (ND) and relative  
127 fitness differences (RFD) by (i) explaining how the method relates to Chesson’s theory, (ii)  
128 showing how the method can be implemented empirically, and (iii) highlighting the method’s  
129 key limitations and assumptions. At the end of Part 1, we use numerical simulations to compare  
130 the five methods to determine if they give the same estimates of ND and RFD, and to assess  
131 whether they yield the same prediction for coexistence based on the invasibility criterion.

132

133 *1.1 Brief review of Chesson's theory*

134           When Chesson first introduced his theory for coexistence, he did not prescribe a specific  
135 empirical approach or experiment that should be used to estimate ND and RFD in real biological  
136 communities. Instead, he used a phenomenological model of competition to show how the  
137 mutual invasibility criterion, a common prerequisite for coexistence, depends on how each  
138 species limits their own growth rate versus that of their competitor (Chesson 1990). Specifically,  
139 he showed that the criterion for mutual invasibility can be expressed as an inequality involving  
140 both ND and RFD (Equation 1).

141  $1 - ND < RFD < \frac{1}{1-ND} \quad (1)$

142 In this inequality, the term  $1-ND$  represents the degree of niche overlap ( $\rho$  in Chesson (1990)),  
143 which ranges from zero when species do not share any resources to one when the resource  
144 requirements of species are identical. RFD represents the ratio of competition-free fitness among  
145 the two species ( $\frac{f_j}{f_i}$  in Chesson (1990)). If this equality is not satisfied, then one of the species is  
146 unable to maintain long-term, positive growth rates and will go locally extinct.

147           Because ND and RFD are not terms that cannot be quantified directly from experiments  
148 or observations, Chesson showed how these forces can be derived from the classic Lotka-  
149 Volterra competition model. In this model, the *per capita* growth rate of species  $i$  is a function of  
150 both intraspecific and interspecific competition as described by Equation 2:

151  $\frac{1}{N_i} \frac{dN_i}{dt} = r_i \left( 1 - \alpha_{ii} N_i - \sum_{j \neq i} \alpha_{ij} N_j \right) \quad (2)$

152 In Equation 2,  $N_i$  is the density of species  $i$ , and  $r_i$  is the intrinsic per capita growth rate of species  
153  $i$ . The intra-specific competition coefficient  $\alpha_{ii}$  describes the *per capita* effect of species  $i$  on the  
154 *per capita* relative growth rate of species  $i$  and is equal to the inverse of the carrying capacity  
155 ( $K_i$ ) for species  $i$ . The inter-specific competition coefficient  $\alpha_{ij}$  describes the *per capita* effect of



156 species  $j$  on the *per capita* relative growth rate of species  $i$ . Equations 3 and 4 relate the inter-  
157 and intra-specific interaction coefficients from the Lotka-Volterra model to ND and RFD:

$$158 \quad ND = 1 - \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}} \quad (3)$$

$$159 \quad RFD = \sqrt{\frac{\alpha_{ij}\alpha_{ii}}{\alpha_{jj}\alpha_{ji}}} \quad (4)$$

160 ND and RFD from Equations (3) and (4) can then be used in Equation 1 to predict coexistence.  
161 Because Chesson derived ND, RFD and the inequality for mutual invasibility based on the  
162 Lotka-Volterra competition model, we use the same approach to explain four of the empirical  
163 approaches described below and show that a fifth approach is ultimately not compatible with  
164 Chesson's derivation.

165

## 166 *1.2 Method based on the parameterized Lotka-Volterra model*

167 1.2.1 Theoretical background. Since Chesson originally used the Lotka-Volterra model to  
168 explain his criterion for coexistence, the most obvious empirical approach for estimating ND and  
169 RFD is to parameterize the Lotka-Volterra competition model (Equation 2) using data collected  
170 from experiments or time-series observations from natural ecosystems. The inter- and intra-  
171 specific interaction coefficients ( $\alpha_{ii}$  and  $\alpha_{ij}$ ) from the Lotka-Volterra competition model can be  
172 used to estimate ND and RFD using Equations 3-4.

173 1.2.2 Empirical approaches. Empirical implementation of the Lotka-Volterra model  
174 requires estimating six different parameters used in Equation 2: intrinsic per capita growth rate of  
175 each species ( $r_i$  and  $r_j$ ), per capita intra-specific competition coefficients ( $\alpha_{ii}$  and  $\alpha_{jj}$ ), and per  
176 capita inter-specific competition coefficients ( $\alpha_{ij}$  and  $\alpha_{ji}$ ). The simplest way to parameterize the  
177 Lotka-Volterra model from experiments would be to use three treatments for each pair of

178 species: a time-series of each species grown alone as a monoculture and one time-series  
179 representing a co-culture of the two species (Figure 1). From each monoculture time series, the  
180 empiricist needs to measure the population density of each species over time, from low density to  
181 steady-state. From these time series, the empiricist can estimate the maximum per capita growth  
182 rate of each species ( $r_i$ ), which occurs as the species' density approaches zero, and the steady-  
183 state population size of each species in monoculture ( $K_i$ ).

184 Empiricists have two options for using the monocultures to estimate the intraspecific  
185 competition coefficients ( $\alpha_{ii}$ ). The first option is to estimate these interaction coefficients using  
186 the slope of each species' relative growth rate (scaled on its maximum growth rate  $r_i$ ) versus its  
187 population density (Figure 1A and 1B, right). This slope has the opposite sign of the intraspecific  
188 interaction coefficient  $\alpha_{ii}$ . However, in practice this slope is unlikely to be fixed across all  
189 population densities. As a result, intra- and inter-specific competition coefficients measured in  
190 competition experiments are likely to vary with population densities (Abrams 1980). An  
191 alternative strategy is to use the assumption that intraspecific competition coefficients ( $\alpha_{ii}$ ) are  
192 equal to  $1/K_i$ , which comes directly from the Lotka-Volterra model in Equation 2. This approach  
193 yields estimates of  $\alpha_{ii}$  that cannot, say, be used in Equation 2 to re-create transient dynamics  
194 observed in time series, but can be used in Equations 3 and 4 to estimate ND and RFD and  
195 predict coexistence using Chesson's inequality (Equation 1).

196 Next the empiricist would perform a competition experiment in which both of the species  
197 are introduced to habitat at low density and the population density of each species is measured  
198 over time (Figure 1C). From these time series, the empiricist must use non-linear regression to  
199 parameterize the interspecific interaction coefficients ( $\alpha_{ij}$  and  $\alpha_{ji}$ ) by substituting the parameter

200 estimates from the monocultures into Equation 2. Finally, the empiricist can use all four  
201 interaction coefficients to compute ND and RFD using Equations 3 and 4.

202 1.2.3 Limitations. A key assumption of this approach is that the intra- and inter-specific  
203 competition coefficients are fixed with respect to population sizes of either species. In other  
204 words, the first individual and the last individual added to a population have the same per capita  
205 effect on the growth rates of its own species or that of its competitor. This assumption is not  
206 always met in real biological communities where intra- and inter-specific competition  
207 coefficients can depend on species' densities (Smith-Gill and Gill 1978). The assumption also  
208 does not apply when the mechanisms leading to competition are driven by non-linear  
209 dependence on resources. Examples include functional responses of consumers to prey density  
210 and non-linear dependence of growth rates on abiotic resource availability (e.g. the Monod  
211 function). Figure A1 in the Supporting Information shows that, when applied to numerical  
212 simulations based on a well-known consumer resource model parameterized with real biological  
213 data, intraspecific coefficients measured in monoculture near equilibrium lead to inaccurate  
214 predictions regarding coexistence. However, when the intraspecific interaction terms are  
215 replaced by  $1/K_i$  the method yields accurate predictions. Therefore, in those situations where  
216 competition coefficients are fixed with respect to population size, or can be measured at low  
217 population sizes of each species, then the empirical approaches can be used to estimate ND and  
218 RFD.

219

220 *1.3 Sensitivity method*

221 The second method for estimating ND and RFD, the sensitivity method, is similar to the  
222 Lotka-Volterra method in that it is phenomenological and requires information from direct  
223 competition experiments.

224 1.3.1 Theoretical background. The sensitivity method quantifies the proportional  
225 reduction in a species' growth rate when invading a steady-state population of its competitor  
226 (Carroll et al. 2011, Narwani et al. 2013). In this method, the maximum growth rate of each  
227 species in monoculture ( $\mu_i$ ) and when invading a steady-state population of the competitor  
228 species ( $\mu_{ij}$ ) are used to calculate each species sensitivity to interspecific competition ( $S_i$ ) using  
229 Equation 5:

$$230 S_i \equiv \frac{\mu_i - \mu_{ij}}{\mu_i} \quad (5)$$

231 Carroll and others (2011) and others have shown that ND is proportional to the geometric mean  
232 of these sensitivity measures, whereas RFD represent variation around the mean:

$$233 ND = 1 - \sqrt{S_i S_j} \quad (6)$$

$$234 RFD = \sqrt{\frac{S_i}{S_j}} \quad (7)$$

235 A species' sensitivity to competition is jointly determined by ND and RFD (Carroll et al.  
236 2011, Narwani et al. 2013). Specifically, greater ND between the two species reduces the impact  
237 of interspecific competition so that  $S_i$  will approach zero. Greater RFD, on the other hand, causes  
238 species to be asymmetrically affected by competition such that one species' sensitivity increases  
239 while the other's decreases. While Carroll and others (2011) verbally argued that this method is  
240 compatible with Chesson's theory, in Appendix A we show explicitly how this method relates to  
241 Chesson's theory and prove that it is identical to Equations 3 and 4 when interspecific interaction

242 coefficients of the Lotka-Volterra model are evaluated only at the steady-state population density  
243 of the resident species.

244 1.3.2 Empirical approaches. The sensitivity method uses a combination of monocultures  
245 and pairwise invasion experiments to quantify the reduction in each species' growth rate caused  
246 by a steady-state population density of the other species at its carrying capacity (Figure 2). The  
247 experiment by Narwani et al. (2013) provides an example for how to implement the sensitivity  
248 method empirically. Their experimental system involved species of freshwater green algae  
249 growing under controlled conditions in the laboratory. They grew each species as a monoculture  
250 in fresh growth medium, starting from low biomass and allowing the populations to reach their  
251 carrying capacity. From these time series, they quantified the per capita maximum growth rate of  
252 each species as a monoculture, which occurs when the focal species is at low density ( $\mu_i$  and  $\mu_j$ ).  
253 After each species reaches its carrying capacity, they introduced the other species from low  
254 density (e.g. 0.01% of  $K$ ) and quantified the per capita growth rate of each species when  
255 invading the other ( $\mu_{ij}$  and  $\mu_{ji}$ ). Finally, for each pair of species, they used these growth rates to  
256 calculate the sensitivity metrics ( $S_i$  and  $S_j$ ) using Equation 5 and used those sensitivity metrics to  
257 calculate ND and RFD using Equations 6 and 7.

258 1.3.3 Limitations. Using the sensitivity method requires one to perform mutual  
259 invasibility experiments, which are only practical for organisms whose population growth rates  
260 can be measured over tractable periods of time. Mutual invasibility experiments are harder to  
261 apply to organisms that grow slowly (e.g. trees) since it would take a long time to acquire time  
262 series of species densities needed to estimate per capita growth rates. Also, it is important to note  
263 that the invasion growth rates ( $\mu_{ij}$  and  $\mu_{ji}$ ) must be measured when the invader population  
264 density approaches zero. Under this condition, intra-specific competition is negligible, and the

265 resident species' density is near steady-state. If the growth rate of the invader species were  
266 measured at greater density of the invader species or lower density of the resident species (i.e.  
267 long after invasion), then the  $S_i$  would be affected by both intra- and inter specific competition.  
268 The resulting predicting regarding species coexistence would be incorrect.

269

#### 270 *1.4 Parameterizing MacArthur's consumer resource model*

271 The third method to estimate ND and RFD from empirical data is to parameterize  
272 MacArthur's consumer-resource model (MacArthur 1970) then use these parameters to calculate  
273 ND and RFD using Chesson's original derivation (Chesson 1990, 2000). This method is different  
274 from both the Lotka-Volterra and sensitivity methods because it does not rely on experiments  
275 where the species are grown together in order to quantify how the species influence each other's  
276 growth rates. Instead, this method works by parameterizing a mechanistic model that describes  
277 species interactions, then reorganizing those parameters to estimate ND and RFD for assessing  
278 Chesson's inequality.

279 1.4.1 Theoretical background. MacArthur's consumer resource model describes how  
280 species consume and thus compete for two or more prey resources (MacArthur 1970). The model  
281 is composed of differential equations representing the growth of each consumer species as a  
282 function of resource densities (Equation 8) and a differential equation (or set) that describes the  
283 population dynamics of each prey resource and their mortality due to consumption by the  
284 consumers (Equation 9).

$$285 \quad \frac{1}{X_i} \frac{dX_i}{dt} = b_i \left( \sum_{l=1}^m c_{il} w_{il} R_l - m_i \right) \quad (8)$$

$$286 \quad \frac{1}{R_l} \frac{dR_l}{dt} = r_l \left( 1 - \frac{R_l}{K_l} \right) - \sum_{i=1}^n c_{il} X_i \quad (9)$$

287 In this model  $X_i$  is population density of the consumer species  $i$  and  $R_l$  is population  
288 density of the prey resource  $l$ . The term  $b_i$  represents the effect of prey consumption on the  
289 growth rate of the consumer,  $r_l$  is the maximum per-capita growth rate of prey resource  $l$ ,  $K_l$  is  
290 the carrying capacity for the prey species  $l$ ,  $w_{il}$  represents the increase in consumer population  
291 density for each unit of prey resource  $l$  consumed. The term  $c_{il}$  is the resource capture rate by  
292 consumer  $i$  on resource  $l$  and  $m_i$  is the density-independent mortality for consumer species  $i$ .  
293 Chesson showed that, by implementing a time-scale separation technique, parameters in  
294 MacArthur's consumer resource model can be used to calculate ND and RFD using Equations 10  
295 and 11 (Chesson 1990, 2000):

$$296 \quad ND = 1 - \sum_l c_{ij} c_{jl} \frac{w_{il} K_l}{r_l} \quad (10)$$

$$297 \quad RFD = \frac{\sum_l c_{jl} w_{il} K_l - m_j}{\sum_l c_{il} w_{il} K_l - m_i} \quad (11)$$

298 The estimates of ND and RFD from this method can then be used to evaluate Chesson's  
299 inequality and predict coexistence (Equation 1).

300 1.4.2 Empirical approaches. Using MacArthur's consumer resource model to estimate  
301 ND and RFD for a pair of species requires quantifying 1) the per capita consumption rate of each  
302 consumer species on each prey resource ( $c_{il}$ , units of prey consumed per unit prey density); 2) the  
303 per capita maximum growth rate and carrying capacity of each prey resource when no consumers  
304 are present ( $r_l$  and  $K_l$ ); and 3) the yield of consumer population density or biomass relative to  
305 each unity of prey consumed ( $w_{il}$ ). Because we are not aware of any empirical studies that have  
306 parameterized the MacArthur model for the purpose of estimating ND and RFD, we describe the  
307 experimental approach that would be required (Figure 3). First, the empiricist would need to  
308 identify or define the prey resources that are available to the consumer species. Each prey  
309 resource would be inoculated or planted at low density into an environment free of other prey

310 resources and consumers, then the population density would be measured over time in order to  
311 estimate the per capita maximum growth rate of the prey ( $r_l$ , which occurs as the prey population  
312 density approaches zero) and its carrying capacity ( $K_l$ , which occurs when the prey growth rate  
313 approaches zero). Next, the experimentalist would need to introduce each consumer species into  
314 several different densities of each prey resource growing as a monoculture. Under those different  
315 prey resource densities, the experimentalist would measure the per capita consumption rate of  
316 prey resource by the consumer species ( $c_{il}$ ) and the yield of consumer density or biomass per unit  
317 prey resource consumed ( $w_{il}$ ).

318         The precise number of parameters to be estimated depends on the number of prey  
319 resources considered by the model. For example, for two consumer species and three prey  
320 resources (Figure 3), the hypothetical experiment requires 18 parameters to be quantified: 3  
321 different maximum per capita growth rates and 3 carrying capacities of the prey resources ( $r_l$  and  
322  $K_l$ ,  $l = 1$  to 3), 6 per capita consumption rates ( $c_{il}$ ;  $i = 1$  and 2,  $l = 1$  to 3), and 6 yields ( $w_{il}$ ;  $i = 1$  and  
323 2,  $l = 1$  to 3). These parameters can then be used in Equations 10 and 11 to obtain ND and RFD,  
324 which can subsequently be used in Equation (1) to predict coexistence.

325         1.4.3 Limitations. The MacArthur's consumer resource model gives a more mechanistic  
326 understanding of competitive interactions among species, and allows one to predict coexistence  
327 for pairs of species without needing to grow them together in a competition experiment.  
328 However, these desirable properties come with greater number of experimental treatments  
329 compared to the Lotka-Volterra and sensitivity methods. In particular, this method requires as  
330 many consumption experiments as there are resources, and each of these experiments involves  
331 measuring consumption rates at a range of resource species densities (Figure 3 B and C). While  
332 this constraint does not impact the ability of the method to predict coexistence under defined



333 conditions, it could limit the extent to which those predictions can be applied to natural  
334 environments where the number of potential prey species is large.

335

### 336 *1.5 Parameterizing Tilman's consumer resource model*

337 Like the method based on MacArthur's consumer-resource model, the method based on  
338 Tilman's consumer resource model does not require species to be grown together in a  
339 competition experiment (Letten et al. 2017). However, unlike the method based on MacArthur's  
340 CRM, the method based on Tilman's CRM is specific to abiotic resources that are controlled by  
341 a constant rate of supply and do not have their own intrinsic growth rate (i.e. a chemostat).

342 1.5.1 Theoretical background. Letten and others (2017) showed how a consumer resource  
343 model (Tilman 1977) can be reorganized to a Lotka-Volterra form in order to estimate ND and  
344 RFD and assess mutual invasibility using Chesson's inequality. This method is based on  
345 Tilman's two-species consumer resource model for two essential and non-substitutable resources  
346 (Tilman 1977). In this model, one set of differential equations describes the growth of each  
347 consumer species as a function of resource availability (Equation 12) and another set of  
348 equations describes the dynamics of abiotic resources and their depletion due to uptake by the  
349 consumer and dilution (Equation 13).

$$350 \quad \frac{1}{N_i} \frac{dN_i}{dt} = r_i \min \left[ \frac{S_i}{S_i + k_{ii}}, \frac{S_j}{S_j + k_{ij}} \right] - D \quad (12)$$

$$351 \quad \frac{1}{R_i} \frac{dS_i}{dt} = D(S_{i0} - S_i) - \sum_{i=1}^n \frac{N_i}{y_{ii}} r_i \min \left[ \frac{S_i}{S_i + k_{ii}}, \frac{S_j}{S_j + k_{ij}} \right] \quad (13)$$

352 In this model,  $N_i$  is the population density of species  $i$ ,  $r_i$  is the maximum per capita growth rate  
353 of species  $i$ ,  $y_{ii}$  is the yield of species  $i$  on resource  $i$ , and  $k_{ii}$  is the half saturation constant for  
354 growth of species  $i$  on resource  $i$ . The term  $S_{i0}$  is the external supply concentration for resource  $i$ ,

355  $S_i$  is the concentration of resource  $i$  in the environment, and  $D$  is equal to both the supply rate of  
356 resources and the density-independent loss rate for both species.

357 Letten et al. (2017) showed how the parameters from Tilman's CRM can be used to  
358 calculate ND and RFD. First, the empiricist must determine which species is limited by each  
359 resource (e.g., using Resource-Ratio theory (Tilman 1982)). This requires comparing the supply  
360 ratio for the two resources against the  $R^*$ s for each species at the pre-determined dilution rate.  
361 As such, the following equations are specific to only the condition where species 1 is limited by  
362 resource 2 and species 2 is limited by resource 1.

$$363 \quad ND = 1 - \sqrt{\frac{y_{12}y_{21}}{y_{11}y_{22}}} \quad (14)$$

$$364 \quad RFD = \sqrt{\frac{\left(\frac{D k_{21} + S_1}{D - r_2}\right)^2 y_{11}y_{21}}{\left(\frac{D k_{12} + S_2}{D - r_1}\right)^2 y_{12}y_{22}}} \quad (15)$$

365 Equations 14 and 15 can be used to estimate ND and RFD and then used in Chesson's inequality  
366 (Equation 1) to predict species coexistence.

367 1.5.2 Empirical approaches. To illustrate how this method could be implemented  
368 empirically, we describe the approach that Tilman first used to parameterize his model (Tilman  
369 1977). In the chemostat environment associated with Tilman's model, the abiotic resources or  
370 nutrients are delivered to the experimental system at a constant supply rate that matches the  
371 density-independent mortality rate (Tilman 1977, 1982). First, he measured the growth kinetics  
372 for two algae species when limited by two different essential resources (silicate and phosphate).  
373 He inoculated each species as a monoculture into growth medium containing a range of  
374 concentrations of the limiting resource (either silicate or phosphate) with all other resource in  
375 excess. From these time series of population densities, he quantified the growth rate of each

376 species as a function of the concentration of the limiting resource following the Monod model  
377 (Figure 4). For each species, this yields estimates of half saturation constants ( $k_{ij}$ ) for each  
378 resource and a single maximum per capita growth rate for both resources ( $r_i$ ). Next, Tilman  
379 quantified the yields ( $y_{ij}$ ) of each species on each resource by measuring the elemental content of  
380 a known number of cells. By following the same approach, an empiricist can quantify the 10  
381 parameters needed to define Tilman's model.

382 As shown by Letten et al. (2017), the parameters described above can be used to predict  
383 coexistence under different resource supply ratios and dilution rates in a chemostat. However,  
384 the form of the equation used to get ND and RFD depends upon the empiricist knowing whether  
385 or not the supply ratio is outside the ratio of  $\frac{R_{ii}^*}{R_{ij}^*}$  and  $\frac{R_{ji}^*}{R_{jj}^*}$  for the two species, as determined by  
386 resource-ratio theory (Tilman 1982).

387 1.5.3 Limitations. The method using Tilman's consumer resource model requires an  
388 empiricist to know precisely which resources the species compete for, which limits its  
389 applicability to many real scenarios and ecosystems where the identity of limiting resources and  
390 the supply rates may not be known. Additionally, the resource supply concentrations must be  
391 fixed and the supply rates must be equal to the density-independent loss rate, which can only be  
392 achieved in certain experimental settings like chemostats. Another important note is that the  
393 elemental content of organisms like algae is not constant and can vary as a function of growth  
394 rate, nutrient availability, and other factors (Sterner and Elser 2002). Non-constant yields due to  
395 luxury uptake and variable internal stores (Grover 1991) have important consequences for  
396 competition, but it has yet to be determined how this plasticity affects estimation of ND and  
397 RFD.

398

399 *1.6 Negative frequency dependence (NFD) method*

400           The final method that we summarize, the negative frequency dependence method (NFD),  
401 has not been proposed as a means of obtaining estimates of ND and RFD that are directly  
402 compatible with Chesson's inequality (Equation 1). In fact, in Supporting Information C, we  
403 explicitly show that the NFD method cannot be used to derive estimates of ND and RFD that are  
404 consistent with Chesson's theory. However, the NFD method can be used to predict coexistence  
405 using the criterion of mutual invasibility. Moreover, the NFD method has been proposed as a  
406 way to interpret stabilizing and equalizing forces from Chesson's original theory, and the method  
407 has been used to illustrate the impacts of ND and RFD in manipulative experiments (Adler et al.  
408 2007, Levine and HilleRisLambers 2009).

409           1.6.1 Theoretical background. The NFD method quantifies the change in the per capita  
410 growth rate of a species as a function of its frequency in a community (Adler et al. 2007, Levine  
411 and HilleRisLambers 2009). Here the frequency of a species refers to the proportion of total  
412 biomass or individuals in a community belonging to that species. This method makes the key  
413 assumption that the community is saturated with respect to total species densities, thus a  
414 frequency of 1 represents a steady-state monoculture at its carrying capacity. At all other  
415 frequencies, the community composition need not be at a steady-state, but it is assumed that any  
416 increase in the population density of one species will be offset by a decrease in population  
417 density of another species. Using this assumption, the slope of the NFD relationship has been  
418 used to reflect the difference between intra- versus inter-specific competition (Adler et al. 2007).  
419 Increasing species  $i$ 's frequency means that individuals of species  $i$  will compete more with  
420 individuals of its own kind than with individuals of other species, and will thus experience  
421 stronger intraspecific competition than interspecific competition. Therefore, if intra-specific

422 competition is greater than inter-specific competition, the species affects its own growth rate  
423 more than it affects the growth rate of other species, and the NFD slope should be negative.

424         The NFD method is most often used as a graphical approach for understanding the  
425 balance of ND and RFD (Figure 5). According to Adler et al. (2007), a more negative NFD  
426 slope represents a stronger stabilizing force, which they argue is proportional to the ND in  
427 Chesson's inequality. Similarly, they argue that the difference between species' growth rate in  
428 the absence of stabilizing forces is the equalizing force, proportional to RFD. In Supporting  
429 Information C we show that this NFD slope is not equivalent to ND and that the difference in  
430 elevation between species' NFD relationships is not RFD. Although the NFD approach does not  
431 yield estimates of ND and RFD that are consistent with Equation 1, this method can still be used  
432 to make predictions about coexistence based on Chesson's mutual invasibility criterion.  
433 Specifically, if each species has a positive growth rate at a frequency approaching 0 then the  
434 mutual invasibility criterion is satisfied, and coexistence is assured. Adler and others (2007)  
435 showed how both the slope and elevation of the NFD plot are needed to accurately determine  
436 whether this condition is met.

437         1.6.2 Empirical approaches. The NFD method could be implemented using either  
438 experiments or observations from natural ecosystems (Figure 5A). Using the experimental  
439 approach, an empiricist could grow both species together and measure their densities over  
440 time. First, the empiricist would need to ensure that total community density or biomass was  
441 saturated. One way to do this would be to use invasion experiments in which the resident species  
442 is grown to steady-state and then the invader species is introduced at low density. Using the  
443 invasion approach guarantees that the community is saturated since any decrease in the resident's  
444 population density is met with an increase in the density of the invader. Next, the empiricist

445 could use the time series for each species' density to calculate the per capita growth rate and  
446 frequency of each species at each time point. Alternatively, the NFD method could be  
447 implemented using observational data from natural ecosystems (Adler et al. 2010). This  
448 approach could allow an empiricist to estimate frequency dependence for species that are not  
449 easily manipulated (e.g. trees). To use this approach, an empiricist would quantify the per capita  
450 growth rate and the relative frequency of the species in different habitat patches or along  
451 ecological gradients. Although this approach has not been applied empirically to make  
452 predictions regarding coexistence (but see Yenni et al. 2017), it is one of only two methods  
453 reviewed here that do not require manipulative experiments.

454       Having obtained pairs of growth rate and frequency from either experiments or  
455 observational studies, the empiricist can construct plots of the NFD relationship (Figure 5B).  
456 This relationship can be used to estimate the growth rate when each species approaches  
457 frequency of zero. If either of the two species does not have a positive growth rate when rare,  
458 then the pair will not coexist. Since the NFD method does not yield estimates of ND and RFD  
459 that can be used in Equation 1, the utility of this method is completely dependent on its ability to  
460 accurately predict mutual invasibility (i.e. growth rates at frequency of 0). In many cases, this  
461 prediction will be based on observations made at species frequencies greater than zero. As long  
462 as the relationship between a species' frequency and its growth rate is linear, the NFD slope and  
463 elevation can theoretically be used to predict whether both species will have positive growth  
464 rates when rare, thus meeting the mutual invasibility criterion. If the NFD relationship is not  
465 linear, then the NFD method can give inaccurate predictions (Figure 5B, see Limitations below).  
466 Indeed, even when applied to numerical simulations from the simple Lotka Volterra model, the

467 NFD slope is not constant across species frequencies in a saturated community (Supporting  
468 Information Figure C1).

469 Levine et al. (2009) demonstrated how the NFD method can be implemented  
470 experimentally. In their study with 10 species of grassland plants, they manipulated the relative  
471 frequency of each focal species by varying the proportion of seeds belonging to the focal species  
472 versus all other species. At the end of the growing season, they quantified the growth rate of each  
473 species in their study plots by multiplying the number of seeds belonging to each species by the  
474 proportion of those seeds that were viable the next year. They then quantified the slope of NFD  
475 by plotting the growth rate of each species against its frequency in the initial community.  
476 Although the slope of NFD is not equal to Chesson's ND and the difference in intercepts is not  
477 equal to RFD, the authors showed that the effect of niche differences on growth rates can be  
478 removed by experimentally maintaining each species' density at a constant, non-equilibrium  
479 level that is not subject to competition from other species. Their experiment showed that  
480 effectively removing niche differences among species (even without measuring them) led to  
481 dominance by the species with the highest per capita growth rates. In other words, in the absence  
482 of ND the outcome of competition was determined by RFD. It is important to note that while this  
483 approach is based on Chesson's inequality, it does not require measuring ND and RFD.  
484 Similarly, other studies have measured the slope of NFD as evidence for the importance of  
485 stabilizing forces, but did not directly interpret the slope as ND or the intercepts as RFD (Yenni  
486 et al. 2017).

487 1.6.3 Limitations. Despite some of the desirable aspects of the NFD method in terms of  
488 empirical approaches (above), it has three key limitations. First, unlike the other four methods  
489 summarized in this paper, the NFD method does not yield estimates of ND and RFD. This may

490 not be a concern if the purpose of the study is simply to predict species coexistence and does not  
491 focus on explaining why certain pairs coexist while other pairs do not. Second, the NFD method  
492 assumes that the community density is saturated across the range of species' frequencies  
493 observed. Meeting this assumption in experiments requires sufficiently long time series to show  
494 that total biomass of a community is fixed. In observational studies based on natural ecosystems,  
495 it might not be possible to ensure that total biomass is saturated.

496         The third limitation of the NFD method is that the relationship between a species'  
497 frequency and growth rate is often non-linear (Figure 5). In Appendix C, we show the NFD  
498 method can lead to incorrect predictions about species coexistence when applied to systems with  
499 non-linear relationships between species' growth rates and densities. If the slope and elevation of  
500 the NFD plot are evaluated over a narrow range of species frequencies, and those data were used  
501 to extrapolate to predict growth rate as frequency approaches zero, then the method could make  
502 inaccurate predictions about mutual invasibility and coexistence. If the relationship between each  
503 species' frequency and its growth rate is not linear, then an empiricist must adequately describe  
504 the relationship to account for the non-linearity. This means that for an empiricist to use the NFD  
505 method, they would need to either 1) measure the growth rate of each species across the full  
506 range of frequencies to establish that the growth rate of each species is linearly related to its  
507 frequency or 2) evaluate the growth rate of each species when rare (i.e. directly demonstrate  
508 mutual invasibility). Both of these options would dramatically increase the effort required but  
509 may be necessary in systems where only observational studies are possible.

510

511 *1.7 Do the methods give the same prediction regarding coexistence?*



512           Although each of the five methods can be used to predict coexistence, the experimental  
513 approaches required for those methods are different, and it is not clear that the methods would  
514 yield the same predictions (or values of ND and RFD) if applied to the same study system. Here  
515 we use numerical simulations to investigate whether four of those methods, when implemented  
516 as shown in Figures 1, 2, 3, and 5, lead to the same prediction regarding coexistence and give the  
517 same estimates of ND and RFD. We could not include both the method based on MacArthur's  
518 CRM and the method based on Tilman's CRM since these mechanistic models have  
519 incompatible assumptions – the resources in MacArthur's CRM have their own population  
520 dynamics while the resources in Tilman's CRM are abiotic and governed by a constant rate of  
521 supply. We chose to use numerical simulation for this demonstration since we are unaware of  
522 any empirical dataset that has been, or could be, analyzed using more than two of the methods.  
523 The numerical simulations were based on Tilman's consumer-resource model (Tilman 1977)  
524 with two species of phytoplankton competing for two essential resources (See Supporting  
525 Information A). For each set of resource conditions, we performed numerical simulations that  
526 represent four distinct methods: 1) fitting the Lotka-Volterra model to monocultures and a co-  
527 culture (Figure 1), 2) the sensitivity method (Figure 2), 3) the method using Tilman's CRM  
528 (Figure 4), and 4) the NFD method (Figure 5).

529           Figure 6 shows that under specific limiting assumptions, all four methods made the same  
530 prediction about coexistence and that these predictions matched the outcome based on the  
531 equilibrium condition from simulations. Across the different resource conditions that we  
532 explored, the two species were predicted to coexist when the resource supply conditions caused  
533 each species to be limited by a different resource, consistent with resource-ratio theory (Tilman  
534 1977). However, this agreement among the methods was conditional on how the Lotka-Volterra

535 and NFD methods were parameterized. The Lotka-Volterra method only matched the predictions  
536 for coexistence from the other methods when we assumed that intraspecific competition  
537 coefficients were equal to the inverse of the carrying capacity (Supporting Information Figure  
538 A1; Section 1.2). When we estimated the intraspecific coefficients directly from the monoculture  
539 time series as they approached their carrying capacity, the method produced incorrect predictions  
540 and overestimated the range of parameter space allowing for coexistence. Similarly, the NFD  
541 method only matched the predictions for coexistence from the other methods when we 1)  
542 evaluated the slope of NFD when species' frequencies were approaching zero and 2) used both  
543 the slope and the intercept to predict the growth rate when frequency approaches zero. Unless  
544 these conditions were met, the NFD method tended to over- or under-estimate the region of  
545 resource conditions that allows for coexistence (Supporting Information Figure A1).

546

#### 547 *1.8 Do the methods yield the same values of ND and RFD?*

548 Although the methods gave the same predictions regarding coexistence, Figure 6 (F and  
549 G) shows that the methods do not yield the same values of ND and RFD, even when applied to  
550 the same simulated study system. The Lotka-Volterra method (using the simplification that  
551  $\alpha_{ii}=1/K_i$ ) and the sensitivity method gave identical estimates of ND and RFD across the range of  
552 resource conditions used, but these estimates differed from the method based on Tilman's  
553 consumer resource model. This disparity can be explained by the fact that the Lotka-Volterra and  
554 sensitivity methods assume that per capita inter- and intraspecific interaction coefficients are  
555 independent of species' densities. Although this assumption is likely to be violated when species'  
556 population dynamics are affected by mechanisms that produce non-linearity between population  
557 densities and growth rates, using the assumption that  $\alpha_{ii} = 1/N_i^*$  allows for accurate predictions

558 regarding coexistence. In contrast, the method based on Tilman's consumer resource model does  
559 not assume that interaction coefficients are independent of species densities, but instead  
560 quantifies both inter- and intraspecific interaction coefficients only at the steady-state densities  
561 predicted for monocultures that undergo invasion. This means that the interaction coefficients,  
562 and thus ND and RFD, measured according to either of the phenomenological methods (Figures  
563 1 and 2) are unlikely to match the values predicted from a mechanistic method, even though both  
564 can correctly predict mutual invasibility.

565         This comparison of methods highlights an important caution, namely that estimates of  
566 ND and RFD obtained by different methods are not always comparable. Therefore, future  
567 syntheses or meta-analyses should not combine studies that measured ND and RFD by different  
568 methods. Even within a single method (e.g. the Lotka Volterra method) there can be substantial  
569 differences in the estimates of ND and RFD depending on the experimental design and how the  
570 interaction coefficients are parameterized. Similarly, estimates of ND and RFD from the  
571 mechanistic methods are dependent on the non-biological parameters used in those models (e.g.  
572 dilution rates). If a future study were to compile these values from different studies without  
573 ensuring that the same assumptions were used throughout, the results and interpretation of the  
574 synthesis would be meaningless.

575

## 576 **Part 2. An Empiricist's Guide to Selecting a Method To Estimate ND and RFD**

577         Having described and compared the foundation of each empirical method, here in Part 2  
578 of the paper we offer practical guidance to help empiricists determine 1) which method(s) are  
579 most appropriate for their study system and 2) how much experimental effort is required for a  
580 given method. To aid our discussion, we have summarized the methods in Table 1, which is

581 organized into three sections. The section of the table labeled ‘Decision Steps’ is a decision tree  
582 that allows an empiricist to identify the most appropriate method for their study system. The  
583 section labeled ‘Method’ directs the empiricist to the key literature needed to implement the  
584 approach. Last, the section of the table labeled ‘Experimental Requirements’ outlines key aspects  
585 of the experiments that are required to use the method.

586

### 587 *2.1 Decision Steps - deciding which method to use*

588         The first section of Table 1, ‘Decision Steps’, uses a sequence of questions about the  
589 study system to help an empiricist identify the most appropriate method for their work. The first  
590 question asks whether the method must yield estimates of ND and RFD that can be directly  
591 related back to Chesson’s inequality (Equation 1). Thus, Question 1 isolates the NFD method  
592 from all others. This distinction is important since the outputs from this method cannot be  
593 compared to the other four methods. However, the NFD method can accurately predict  
594 coexistence based on mutual invasibility and, depending on the answers to the remaining  
595 questions, it could be the most appropriate method for certain study systems. In particular, the  
596 NFD method is the only option that does not require an empiricist to grow each species alone as  
597 a monoculture (Question 2). This could be an advantage for study systems where experimental  
598 manipulations are not feasible (e.g. long-lived species, protected habitats). Several empirical  
599 studies have successfully implemented the NFD method in experiments (Levine and  
600 HilleRisLambers 2009, Chung and Rudgers 2016), and other similar studies have used NFD in  
601 observational studies (Adler et al. 2010).

602         The next question in the ‘Decision Steps’ is whether the empiricist knows which specific  
603 resources the species are competing for and can quantify the dependence of each species’

604 population dynamics on those resources (Question 3). This question separates the four methods  
605 for estimating ND and RFD into two separate groups. The phenomenological methods (Lotka-  
606 Volterra and sensitivity method) are those that are informed by directly quantifying species  
607 interactions, but which make no assumptions about the resources that species are competing for  
608 (highlighted in green). The mechanistic methods based on MacArthur's CRM or Tilman's CRM  
609 assume that species interact only by competing for shared resources (highlighted in blue). To use  
610 either of the mechanistic methods, an empiricist needs to know which resources define niche  
611 differences. In certain cases, it will not be possible for an empiricist to answer 'yes' to Question  
612 3, because the resources required for species to grow are either not known or cannot be readily  
613 quantified (e.g. non-essential resources). When one cannot answer yes to Question 3, then the  
614 Lotka-Volterra and sensitivity methods may be appropriate because they can still quantify ND  
615 and RFD even if the empiricist does not have a good understanding of which resource(s) species  
616 are competing for, and thus, which resources define their niche. Because Question 3 is so  
617 consequential, the remaining steps are particular to either the phenomenological or mechanistic  
618 methods.

619         Deciding between the Lotka-Volterra method and the sensitivity method  
620 (phenomenological methods, highlighted in green), depends on the answers to whether the  
621 method must work for observational datasets (Question 4), whether it is necessary to  
622 experimentally grow each species as at steady-state near its carrying capacity (Question 5), and  
623 whether the method can be used to predict coexistence among 3 or more species simultaneously  
624 (Question 7). An empiricist working with long-lived species or in protected habitats would likely  
625 answer 'observational' to Question 4, eliminating the sensitivity method. In this case, the  
626 empiricist would need to decide whether it is essential to obtain values of ND and RFD

627 compatible with the other four methods (requiring the Lotka Volterra method) or whether the  
628 NFD method could be employed to predict mutual invasibility and thus coexistence. Similarly, if  
629 an empiricist is unable to answer ‘yes’ to Question 5, she/he would be forced to use either the  
630 Lotka Volterra method or the NFD method. Question 5 could be particularly important for  
631 studies performed using slow-growing species where it is possible to parameterize the carrying  
632 capacity term from a time series of species densities, but it would take too long for the species to  
633 approach the carrying capacity to justify beginning an invasion by the other species. Lastly, the  
634 two phenomenological methods differ in terms of whether they can predict species coexistence  
635 among three or more species simultaneously (Question 7). While the Lotka-Volterra model can  
636 be parameterized to obtain all pairwise competition coefficients for a pool of species, it has not  
637 been applied to predicting coexistence of more than two species simultaneously. The sensitivity  
638 method can be used beyond pairwise species interactions (Carroll et al. 2011); however, doing so  
639 is limited to situations where all non-focal species can be considered in aggregate (e.g. species  $i$   
640 invading a community of  $j + k + l$ ).

641         Deciding between the MacArthur and Tilman CRM methods (mechanistic methods,  
642 highlighted in blue), is straightforward and depends on whether the resources that the species  
643 compete for are abiotic and governed by a constant rate of supply (e.g. inorganic nutrients  
644 consumed by plants) or biotic with their own population dynamics (Question 6). It is worth  
645 noting that Tilman’s  $R^*$  concept has been extended to include competition mediated by predators  
646 (e.g.  $P^*$ , (Tilman 1982)). However, to date, models including both predation and abiotic resource  
647 competition have not been related to Chesson’s ND and RFD. Additionally, MacArthur’s  
648 consumer resource model can theoretically work for more than two species at a time, but this has  
649 not been demonstrated for the method based on Tilman’s consumer-resource model (Question 7).

650           Using this decision tree, an empiricist can determine which method(s) are appropriate for  
651 their study system. Depending upon the study system or experimental constraints, an empiricist  
652 may have multiple options for which method to use. In these cases, it can be useful to consider  
653 the experimental requirements of each method (Table 1, *Experimental Requirements*) and the  
654 tradeoffs among the methods in terms of their utility as discussed in Part 3.

655

## 656 *2.2 Experimental requirements*

657           In addition to the ‘Decision Steps’ outlined in Table 1, there are important practical  
658 differences for the experimental or observational studies needed to quantify ND and RFD for  
659 each method. The most important difference in study design among these methods is whether or  
660 not they require the species to be grown together in order to make a prediction about coexistence.  
661 The NFD method and the two phenomenological methods require each pair of species to be  
662 grown together in at least one co-culture, but the mechanistic methods do not require these co-  
663 cultures. This distinction means that only the mechanistic methods can be used to make  
664 predictions about coexistence of species without performing pairwise competition experiments or  
665 analyzing time series from co-cultures. For example, consider a typical competition experiment  
666 involving a pool of three species (A, B, and C). The mechanistic methods can make predictions  
667 about species coexistence for all pairwise combinations of the species (A+B, A+C, and B+C)  
668 based solely on information about each species when grown individually. The phenomenological  
669 methods, however, require at least one co-culture for each pairwise combination of species,  
670 which means that information from monocultures and pairs A+B and A+C cannot be used to  
671 make any prediction about coexistence for the pair B+C.

672           The need for species to be grown together in co-culture has important implications for the  
673 total number of experimental treatments that would be required to quantify ND and RFD.  
674 Depending on the study design, experiments using the phenomenological methods can require  
675 more experimental treatments to predict pairwise coexistence among a pool of species than the  
676 mechanistic methods do. For the phenomenological methods, the number of experimental  
677 treatments required for all pairwise combinations of species increases exponentially with each  
678 additional species being considered. In contrast, for the mechanistic methods the total number of  
679 experimental treatments required increases linearly with the number of species being considered.  
680 This is because the methods based on consumer-resource models do not require any direct  
681 competition experiments in order to estimate competition coefficients ( $\alpha_{ij}$ ), while all of the  
682 phenomenological methods require at least one co-culture for each species pair (and often more  
683 than one) in order to quantify the competition coefficients. As a result, the relative efficiency of  
684 the phenomenological versus mechanistic methods depends upon both the number of species  
685 being considered and also the number of resources. When the number of species being  
686 considered is small and the number of limiting resources is few, the difference in experimental  
687 effort can be modest. For example, to predict pairwise coexistence among a pool of four species,  
688 using the sensitivity method requires 16 experimental treatments (time series): 4 monocultures to  
689 parameterize both maximum growth rate and carrying capacity and 12 invasions to parameterize  
690 sensitivity (A invading B, B invading A, etc.). In contrast, using either of the consumer resource  
691 models (two limiting resources) would require two experiments per species for a total of 8  
692 experiments. If the mechanistic methods require parameterizing four or more limiting resources,  
693 then the phenomenological methods may be more efficient for a pool of four species. However,  
694 for larger pools of species the difference can be substantial. Obtaining pairwise estimates of ND



695 and RFD for a pool of 10 species requires between 55 and 180 treatments for the  
696 phenomenological methods but as few as 20 treatments for mechanistic methods.

697 In addition to the number of experimental treatments required for each method, it is  
698 important to consider the amount of effort and time required for each experimental treatment.  
699 Specifically, the NFD, Lotka Volterra, and sensitivity methods require time series of species  
700 densities in the experimental or observation study. In the case of the NFD and sensitivity  
701 methods, these time series may be short in duration (i.e. at least one generation) and focused only  
702 on population dynamics when species densities are very low or near the steady-state density of  
703 monocultures. However, the Lotka-Volterra method requires longer time series in order to  
704 parameterize both the interaction coefficients and carrying capacities. Longer time series in  
705 monoculture and co-culture are more easily attainable for quickly-growing species like microbes  
706 and invertebrates, but even short time series could be prohibitively arduous for slowly growing  
707 species like trees.

708 Ultimately, the total effort and resources required for a study is jointly determined by the  
709 method, number of species, number of limiting resources (if applicable), length of time series,  
710 level of replication, and any other design elements. Using Table 1 as a guide, an empiricist  
711 should be able to select a method and begin to design a study that satisfies their aims.

712

### 713 **Part 3. Tradeoffs Among Methods and Suggested Future Directions**

714 Having explained how to select and implement the five methods, we end the paper by  
715 offering some advice for empiricists about how to navigate tradeoffs among the methods, how to  
716 compare and synthesize measurements of ND and RFD from different methods, and lastly, key  
717 future directions for implementing modern coexistence theory empirically.

718

719 *3.1 Tradeoffs between phenomenological and mechanistic methods*

720         Given the substantial differences in experimental design requirements and effort that are  
721 required to execute the five methods described in Part 1 of the paper, it is highly likely that  
722 empiricists will face choices that require tradeoffs when selecting a particular method for their  
723 study system. The most obvious and important tradeoffs occur between the phenomenological  
724 methods and the mechanistic methods, which differ in three important ways. First, the  
725 phenomenological methods (i.e. the NFD, Lotka-Volterra, and sensitivity methods) make no  
726 assumptions about the resources that species compete for. This could be beneficial for  
727 empiricists who can still measure ND and RFD even if they lack detailed information about the  
728 biological resources that species compete for. But the trade-off for this lack of knowledge is the  
729 need for pairwise experiments to directly quantify ND and RFD. Second, a key disadvantage of  
730 all three phenomenological methods is that they require each pair of species to be grown together  
731 in competition, which causes the total effort to increase exponentially as more species are  
732 considered. Third, the results of phenomenological experiments are specific to each pair of  
733 species tested and cannot be generalized to interactions beyond that pair. Furthermore, the  
734 predictions from the phenomenological methods are specific to the exact environmental  
735 conditions, like resource density or resource supply rates, used in that experiment and cannot be  
736 generalized outside of those same conditions.

737         But for those empiricists who can identify the resources that species compete for, use of  
738 the mechanistic methods allows for potentially fewer experiments that are more easily  
739 generalized to predict coexistence among all species in the focal species pool. Indeed, an  
740 empiricist who is able to answer ‘yes’ to Question 3 in Table 1 could use a mechanistic method

741 to predict coexistence (or not) for not only the species pair of interest, but any and all species  
742 pairs of interest based solely on experiments that are performed with each species grown alone in  
743 monoculture. Importantly, the mechanistic methods also offer the ability to make predictions  
744 about species coexistence under different environmental conditions. For example, Letten et al.  
745 showed that the Tilman consumer resource model can be used to predict the ND and RFD at  
746 different nutrient supply concentrations or dilution rates (Letten et al. 2017). The ability of the  
747 mechanistic methods to handle some changes to environmental context, while limited, could be  
748 useful for predicting how anthropogenic stressors (e.g. nutrient pollution) are likely to affect  
749 species coexistence. The ability to make predictions about combinations of species without the  
750 need to perform all pairwise competition experiments has already been touted as a benefit of the  
751 mechanistic models (Tilman 1982), and it could be useful for addressing certain ecological  
752 questions that do not always lend themselves well to manipulative experiments (e.g. invasions by  
753 introduced species, coexistence of rare or endangered species).

754

### 755 *3.2 Comparing and synthesizing measurements of ND and RFD*

756 To date, only three of the four methods proposed for measuring niche and relative fitness  
757 differences have been used empirically. No one, to our knowledge has used the MacArthur  
758 consumer-resource model to quantify ND and RFD in any real system, despite publications  
759 showing that it is possible. That means that most of our inferences about ND and RFD that have  
760 been measured empirically stem from the phenomenological methods. Furthermore, we are  
761 unaware of any study that has applied more than one method to the same empirical study system.  
762 As such, we have no way to compare the performance of the methods empirically. Therefore, we  
763 believe an important avenue for future research is to focus on measuring ND and RFD using

764 more mechanistic models, and for studies that measure ND and RFD using different methods in  
765 the same study system so that we can compare results and attempt to demonstrate equivalence or  
766 non-equivalence of these methods.

767         Even as we call for more mechanistic experiments and comparative studies, we caution  
768 against the inevitable urge to synthesize ND and RFD in an informal data synthesis or more  
769 formal meta-analysis. Although we have shown that all five existing methods should correctly  
770 predict the qualitative outcome of coexistence, the methods are by no means mathematically or  
771 practically equivalent. In some cases the methods will not yield the same ND and RFD, even  
772 when applied to the same species and environmental conditions. Indeed, given the differences in  
773 how the methods are implemented (Figures 1-5), there is no reason to expect, *a priori*, that the  
774 quantitative values of ND or RFD measured for a particular group of organisms using one  
775 method will produce quantitatively similar values of ND (or RFD) for that same group of  
776 organisms using a different method. As such, the methods are not directly comparable, and the  
777 measurements they produce should not be mixed-and-matched to produce some synthesized  
778 estimate of the niche or fitness difference for, say, grassland plants.

779

### 780 *3.3 Future directions for implementing modern coexistence theory*

781         In our view, there are at least two important new directions that work on species  
782 coexistence must go if Chesson's modern coexistence theory is to become widely implemented  
783 and more practical. First, each of the empirical methods described in this review are focused on  
784 fluctuation-independent mechanisms. To also include fluctuation-dependent mechanisms of  
785 coexistence in Chesson's framework, we need to expand the scope of the five methods reviewed  
786 here or even develop new empirical methods. To our knowledge, there have been limited

787 empirical studies that explicitly quantify the fluctuation dependent mechanisms, i.e. relative  
788 nonlinearities and storage effects (but see (Angert et al. 2009, Letten et al. 2018)). Even so, it is  
789 well-known that environmental fluctuations mediate species coexistence in some empirical  
790 systems (Caceres 1997, Jiang and Morin 2007) and any modern theory of coexistence is  
791 incomplete without them. It is also important to note that all of the methods developed to date are  
792 only applicable to competitive communities, and cannot be applied to cases where species  
793 facilitate the growth of each other (but see (Bimler et al. 2018)).

794         Second, empirical studies on coexistence need to move beyond prediction of pairwise  
795 species interactions. Several authors have recently emphasized that modern coexistence theory is  
796 under-developed for multi-species systems (Carroll et al. 2011, Levine et al. 2017). In theory, the  
797 pairwise competitive hierarchy between species  $i$  versus  $j$  and  $j$  versus  $k$  might not directly  
798 translate to species  $i$  and  $k$ , particularly when these species are engaged in intransitive  
799 competition or higher-order interactions (Levine et al. 2017). In fact, none of the three  
800 phenomenological methods (the NFD, Lotka-Volterra and sensitivity methods) can deal with  
801 intransitive competition or higher-order interactions. Importantly, the emphasis to date on  
802 pairwise interactions and experimentation means that intransitive competitive interactions and  
803 higher-order interactions, if present, are unaccounted for in our understanding. Chesson's  
804 coexistence framework has been a major advance for understanding coexistence among pairs of  
805 species, and how to expand this framework to multi-species systems should be a priority for the  
806 field.

807

808 **Author Contributions**

809 All three authors designed the synthesis and wrote the manuscript, FHC performed the analytical  
810 derivations, CMG wrote the numerical simulation code and drafted the conceptual figures.

811

## 812 **Supporting Information**

813 In the supporting information section we provide: (A) Numerical Simulation of Experiments To  
814 Measure ND and RFD and Predict Coexistence, (B) Relating the Sensitivity Method to  
815 Chesson's Definition of ND and RFD Using the Lotka Volterra Model, and (C) Relating the  
816 Negative Frequency Dependence Method to Chesson's ND and RFD. Annotated computer code  
817 is provided as a separate file.

818

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- 902

Decision Steps:	1: Must the method yield estimates of ND and RFD (i.e. Chesson's inequality)?	No	Yes	Yes	Yes	Yes
	2: Can you grow each species as a monoculture?	Yes or No	Yes	Yes	Yes	Yes
	3: Do you know the resources the species are competing for?	No	No	No	Yes	Yes
	4: What type of study will be used to parameterize the model?	Experimental or Observational	Experimental or Observational	Experimental	Experimental	Experimental
	5: Does the method require $\geq 1$ species at steady-state near its carrying capacity?	No	No	Yes	No	No
	6: Is the resource abiotic (non-dynamic), biotic (dynamic), or undefined?	Undefined	Undefined or biotic (dynamic)	Undefined	Biotic (dynamic)	Abiotic* (non-dynamic)
	7: Can the method predict coexistence among 3 or more species simultaneously?	Yes	No	Yes	Yes	No
Method	Negative frequency dependence		Lotka-Volterra	Sensitivity	MacArthur's CRM	Tilman's CRM
	Foundational paper for model	Rees and Westoby 1997	Volterra 1928	MacArthur 1970	MacArthur 1970	Tilman 1977
	Theoretical paper linking model to MCT	Adler et al 2007	Chesson 2000	Carrol et al 2011	Chesson 1990	Letten 2017
	Empirical paper using model for MCT	Yenni et al 2017	Godoy and Levine 2014	Narwani et al 2013	none	Letten 2017
Experimental Requirements	Does the method require the species to be grown together?	Yes	Yes	Yes	No	No
	How many experimental treatments required to measure ND and RFD for all pairs of n species?	$m \cdot n \cdot (n-1)$ , where $m \geq 2$	$n + [n(n-1)/2]$	$n^2$	$m \cdot n$ , where m= number of resources	$m \cdot n$ , where m= number of resources
	Are time-series of species densities required?	Yes, $\geq 1$ generation	Yes, enough generations to approach k	Yes, $\geq 1$ generation	No	No

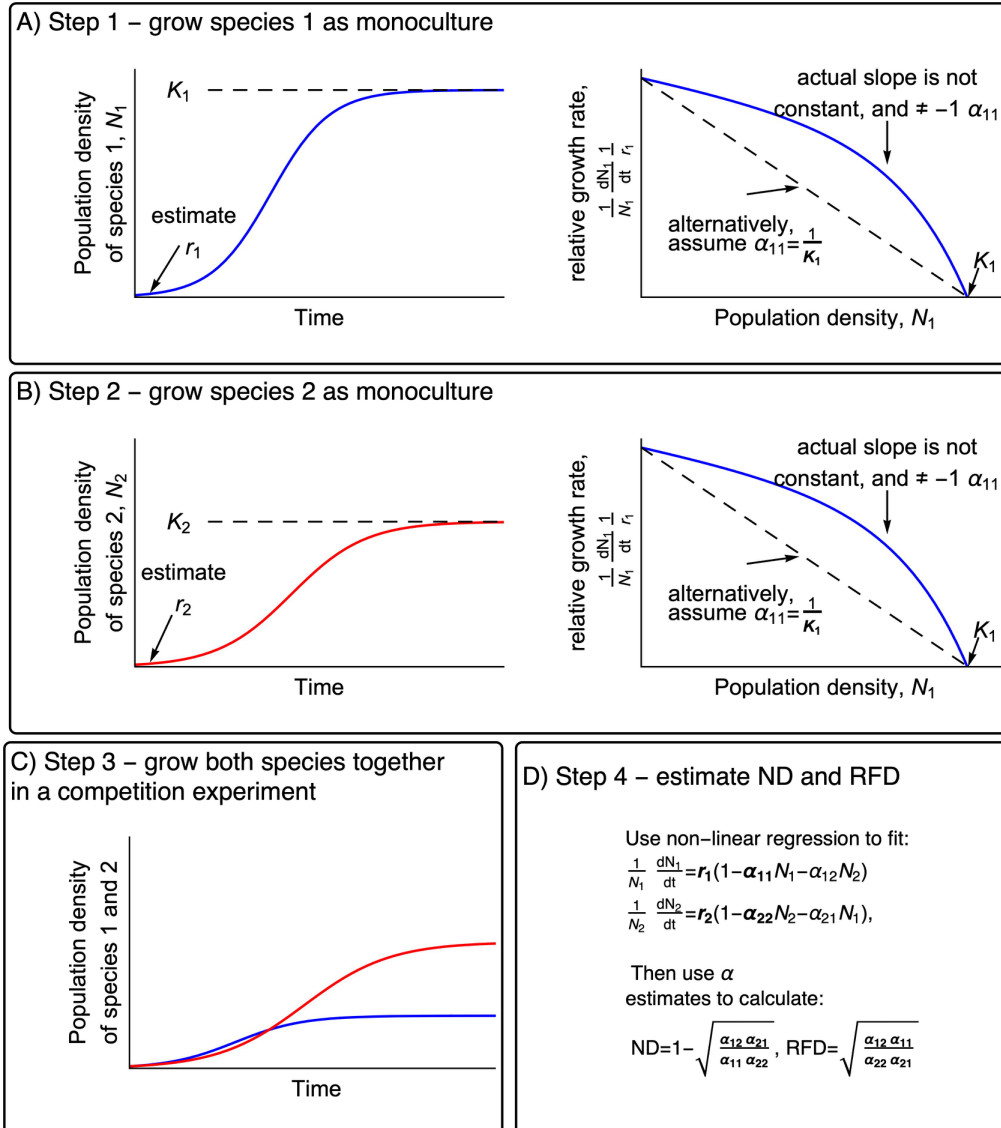
905 Table 1. A practical guide to help empiricists determine which method(s) are most appropriate for a given study system and question.

906 See Part 2 for a step-by-step explanation of this guide. \* While consumer-resource models that include a second trophic level (e.g.

907 predators, P\*) have been developed and used empirically, these approaches have not been linked to ND and RFD.

908

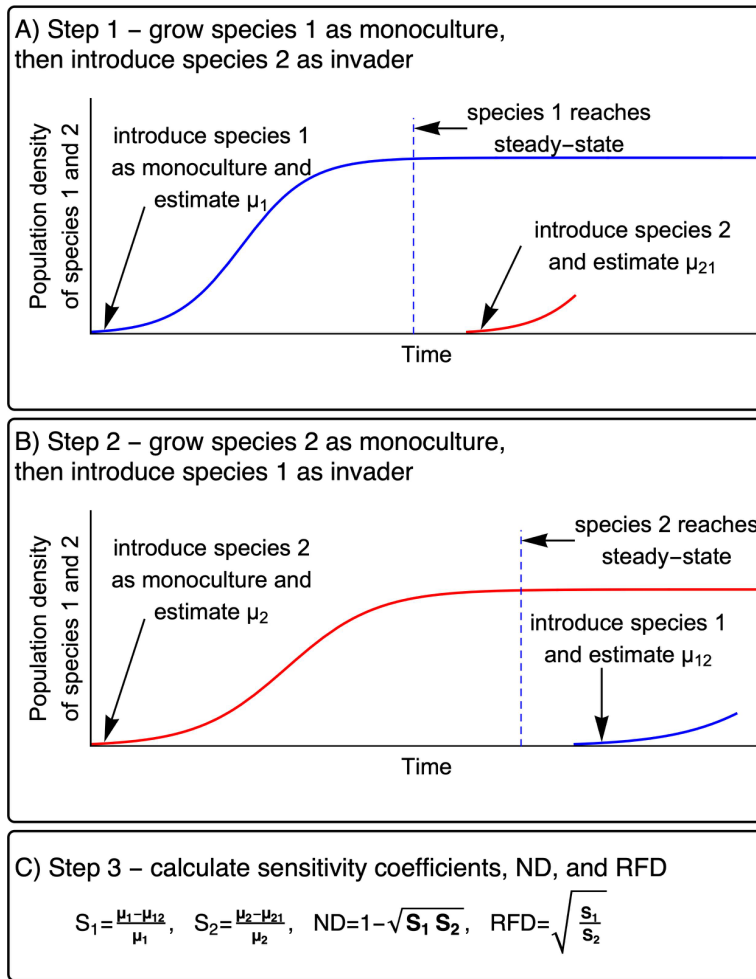
## Figures



909

910 Figure 1. Conceptual plots illustrating how to use the Lotka-Volterra method to estimate ND and  
 911 RFD for use in Chesson's inequality (Equation 1). In each panel, unknown parameters are  
 912 displayed in regular typeface and previously-estimated parameters are listed in bold typeface. In  
 913 panels a and b, the left-hand plots show the time course of the experiment. In panels a and b the  
 914 right-hand plots show the relative growth rate as a function of population density – the slope of  
 915 this relationship is equal to the intraspecific competition coefficient (sign reversed).

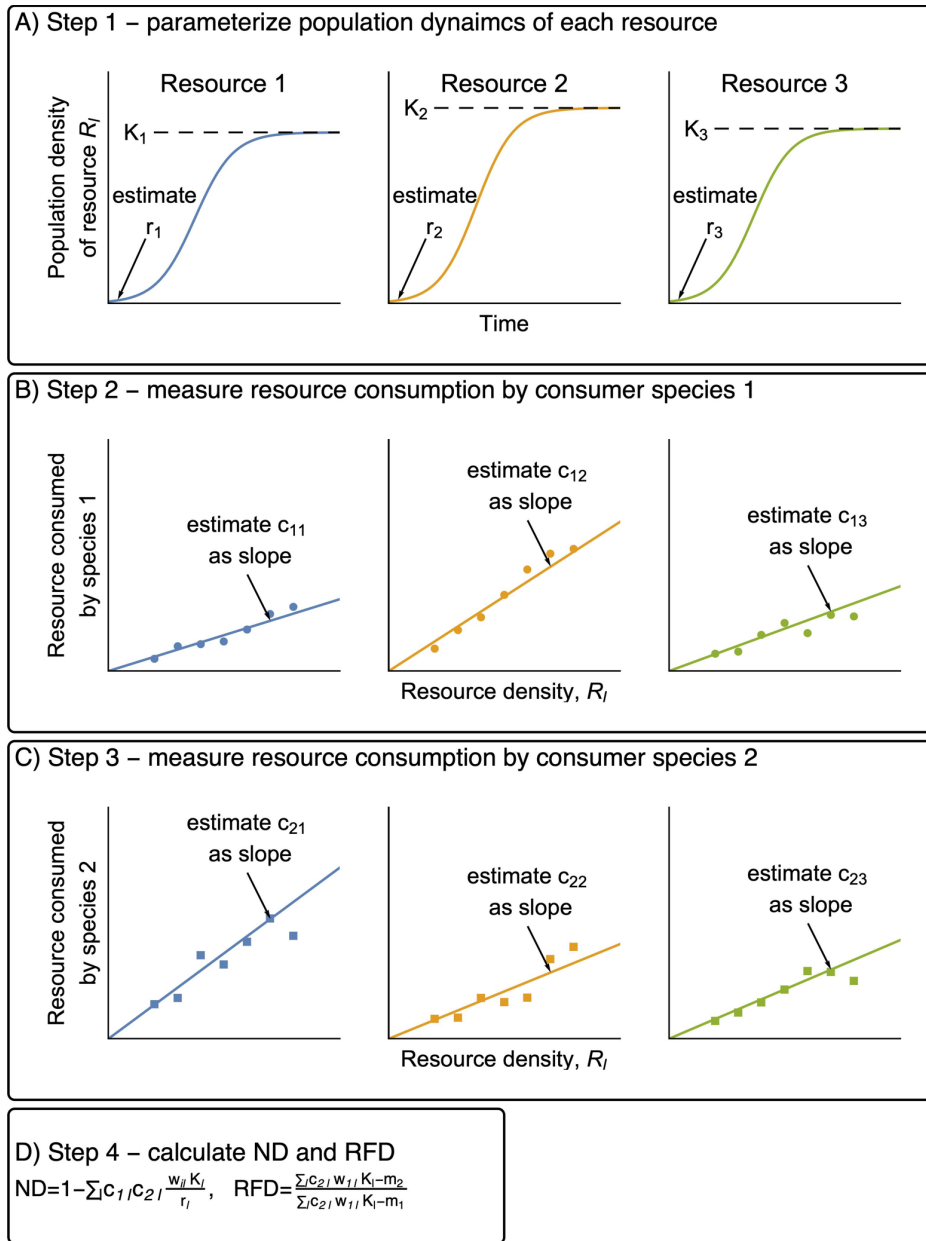
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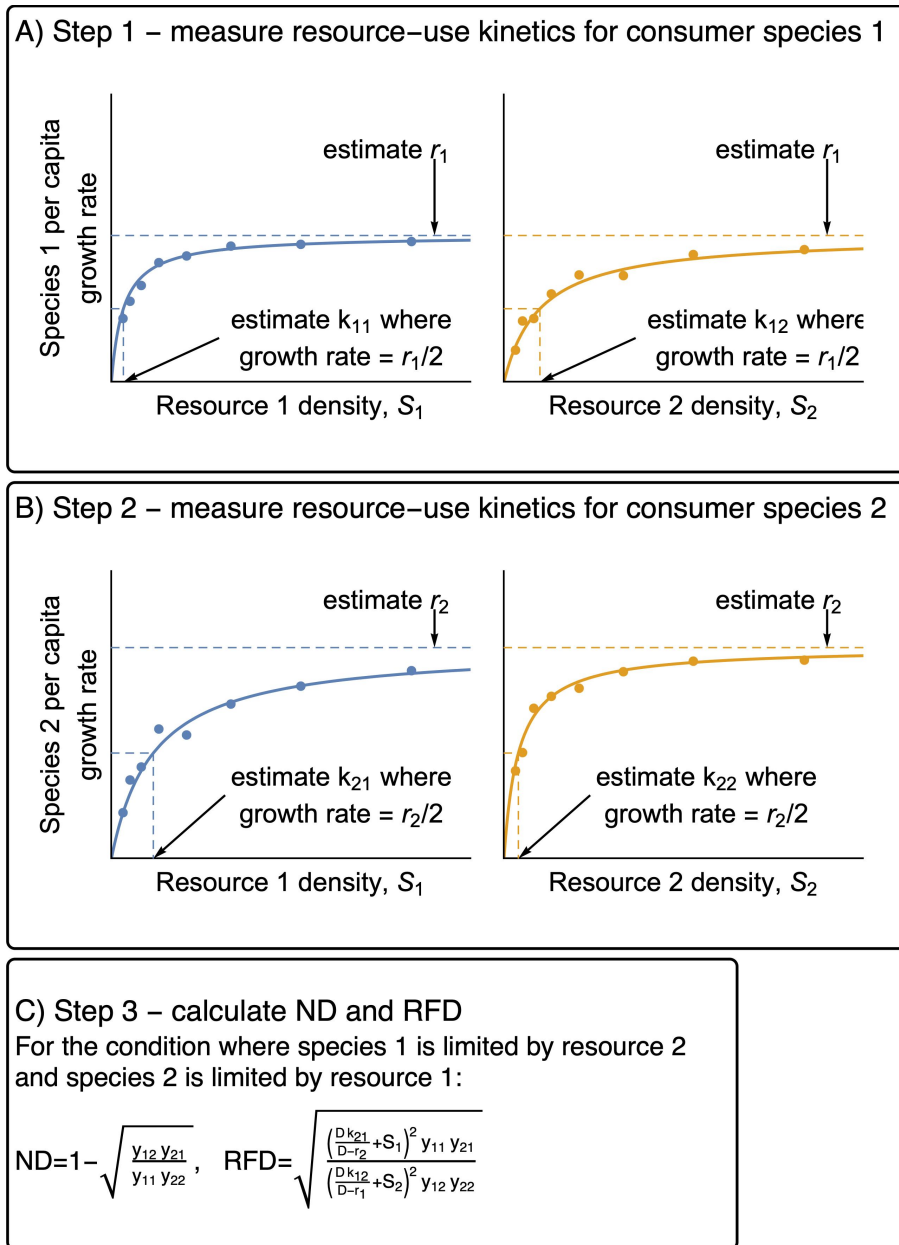
918 Figure 2. Conceptual plot depicting how to implement the sensitivity method in an experiment.

919



920

921 Figure 3. Conceptual plots depicting how the method based on Mac Arthur’s CRM could be  
 922 implemented. The yield term ( $w_{ij}$ , increase in consumer units per unit prey resource consumed),  
 923 can be estimated by measuring these changes for each combination of consumer and resource.



924

925 Figure 4. Conceptual plot depicting how to parameterize the method based on Tilman's

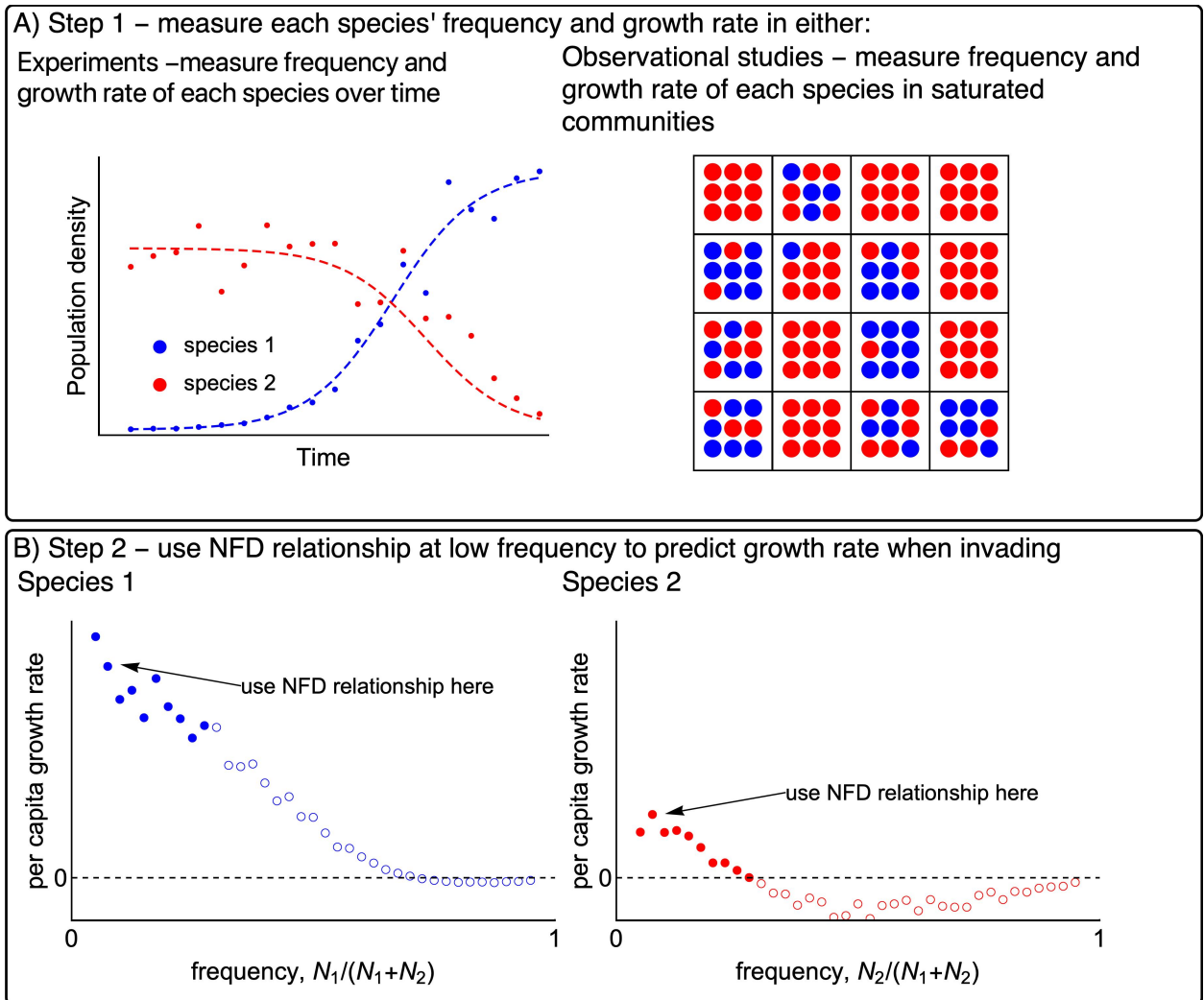
926 consumer resource model. Panels A and B show the experiments needed to parameterize the

927 maximum growth rates and Monod half-saturation constants for growth on each resource,

928 separately for each species. The yield of each species on each resource ( $y_{ii}$ ) can be estimated by

929 measuring the amount of resource consumed by a known number of individuals.

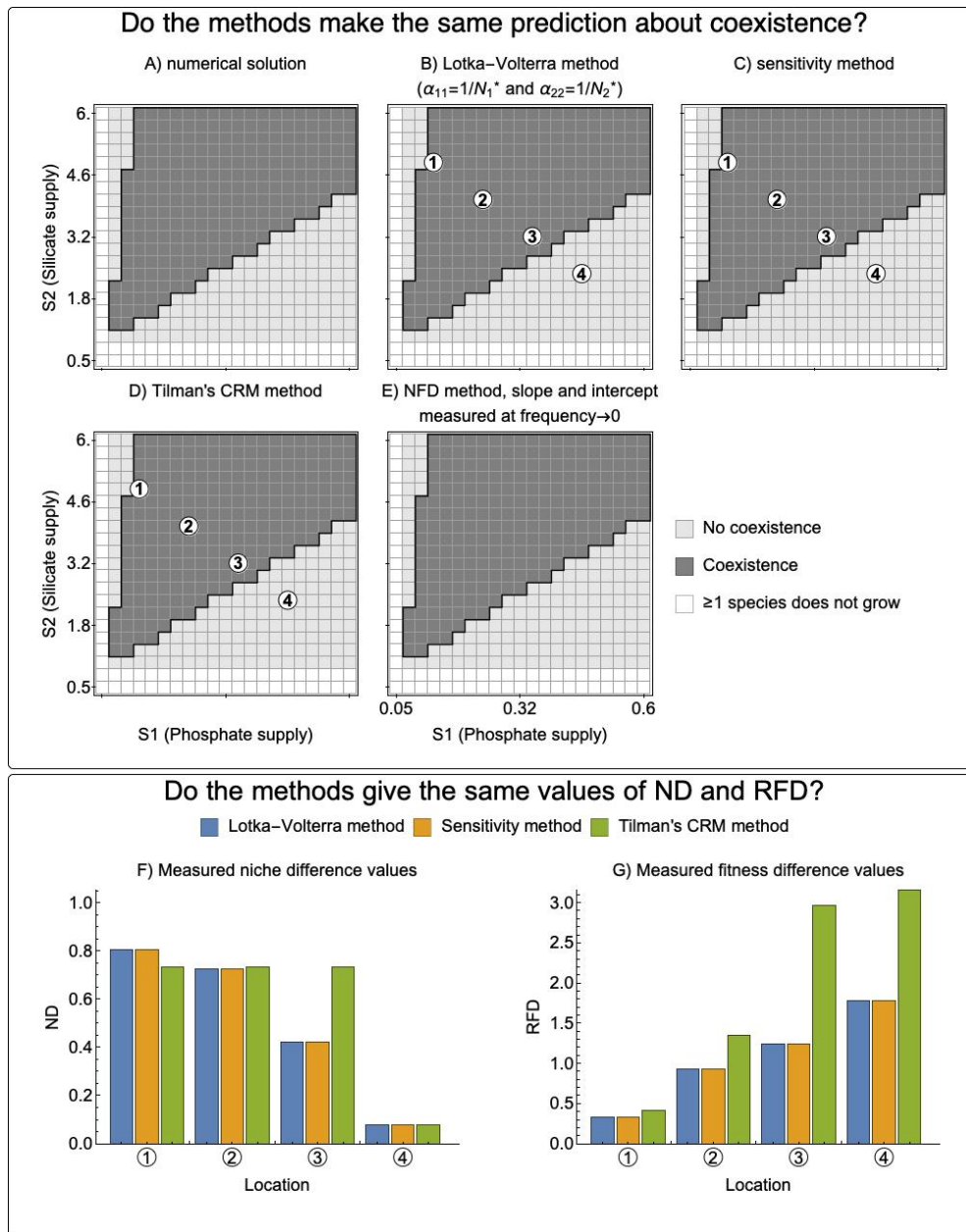
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931

932 Figure 5. Conceptual diagram depicting how the NFD method could be implemented, either  
 933 through and experiment or using observational data from different communities. Panel A shows  
 934 two options for obtaining pairs of each species' frequency and its growth rate in a saturated  
 935 community. The first option is to track population densities over time in one or more competition  
 936 experiments. The Second option is to obtain pairs of frequency and growth rate from different  
 937 Communities or habitat patches in a natural ecosystem. Panel B depicts how the data from either  
 938 experiments or observational studies would be used to estimate the growth rate when frequency  
 939 approaches zero.

940



941  
 942 Figure 6. Comparison of four methods for predicting coexistence and estimating ND and RFD.  
 943 The methods were compared using simulations based on Tilman's parameterized CRM  
 944 (Supporting Information A). In panels A-E, the predictions for coexistence are compared against  
 945 the coexistence outcome based on numerical simulation. White shading means that at least one  
 946 species does not grow under that combination of resource supply concentrations, light gray  
 947 shading indicates that the method predicts that the species will not coexist, and dark shading



948 indicate indicates that the model predicts that the species will coexist. All four of the methods  
949 give the correct predictions regarding coexistence across this region, but this is conditional on  
950 specific limiting assumptions for the Lotka-Volterra and NFD methods (see Supplement C). The  
951 methods did not give the same values for ND and RFD (f and g). The labeled locations in panels  
952 F and G correspond to marked locations in panels b-d and show that the disagreement among the  
953 methods is smaller toward the center of the parameter space that allows for coexistence. The raw  
954 RFD values from the sensitivity method were converted to the same ordering as used in the other  
955 methods (species  $i$  in the denominator rather than the species with the greater sensitivity).  
956 Because the NFD method cannot be used to produce values of ND and RFD that are comparable  
957 with the other four methods, only the predictions regarding coexistence are plotted.

958

959

## 960 **Supporting Information A: Simulation of Experiments To Measure ND and RFD and** 961 **Predict Coexistence**

962 In this supplement, we performed numerical simulations to compare the outcomes from  
963 three methods for measuring ND and RFD and also the NFD method for predicting coexistence.  
964 We used Tilman's parameterized consumer-resource model for two species of phytoplankton  
965 competing for essential and non-substitutable resources (Tilman 1977). Annotated code for the  
966 simulations is provided in a supplemental file. Simulations were performed using the function  
967 NDSolve in Mathematica 11.2 (Wolfram Research), employing a variable step size. For each set  
968 of resource supply concentrations, we performed four simulations: (1) species 1 as a  
969 monoculture, growing from rare to near its equilibrium density; (2) species 2 as a monoculture,  
970 growing from rare to near its equilibrium density; (3) species 1 at its equilibrium density, with  
971 species 2 invading from rare; (4) and species 2 at its equilibrium density, with species 1 invading  
972 from rare. Additionally, we performed numerical simulation where both species are introduced at  
973 low densities and asked whether they coexist at the equilibrium. For each set of simulations, we  
974 manipulated the supply concentration of the two resources in order to determine whether the  
975 methods consistently agree.

976 We implemented the Lotka-Volterra method using information from all four simulations  
977 described above. Simulations 1 and 2 were used to estimate  $r_i$ ,  $K_i$ , and intraspecific interaction  
978 coefficients  $\alpha_{ii}$ . We estimated the intraspecific interaction terms using two different approaches  
979 (Section 1.2). First, we estimated  $\alpha_{ii}$  as the slope of the relative growth rate versus population  
980 density (sign reversed) as the monoculture simulations approach equilibrium (Figure 1).  
981 Alternatively, we used the assumption that  $\alpha_{ii} = 1/K_i$ . We then used the parameter values from the  
982 monocultures, along with simulations 3 and 4, to solve Equation 2 when each species is at low

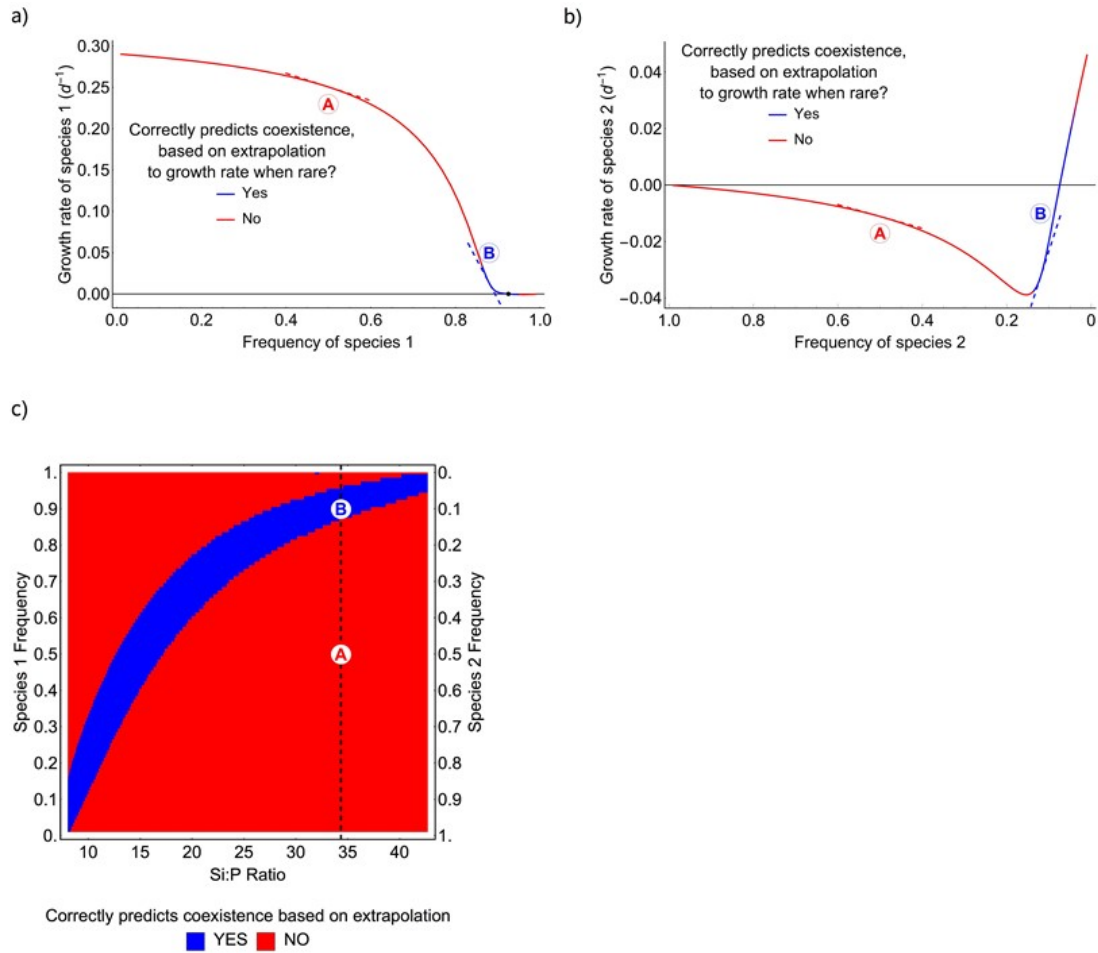
983 density and the other is near equilibrium. We used all four interaction coefficients to calculate  
984 ND and RFD using Equations 3 and 4. We implemented the sensitivity method following  
985 Equations 5 through 7, using output from all four simulations. The raw RFD values from the  
986 sensitivity method were converted to the same ordering as used in the other methods (species  $i$  in  
987 the denominator rather than the species with the greater sensitivity).

988         Next, we compared the four methods including the sensitivity method, the method based  
989 on Tilman's CRM, the Lotka-Volterra method, and the NFD method using the numerical  
990 simulations described above. Under specific assumptions, the methods gave the same prediction  
991 regarding coexistence (Figure A2), though the methods did not produce consistent estimates of  
992 ND and RFD (Figure 6).

993         As described in Appendix C, the NFD method cannot be used to get ND and RFD  
994 estimates that are consistent with the other methods, but nonetheless this method can be used to  
995 predict coexistence based on the same criterion. However, as shown in Figure 5 (using the  
996 Lotka-Volterra model), accuracy of the NFD method depends on the range of frequencies used to  
997 get the slope and elevation. To illustrate how the non-constant NFD slope is problematic in  
998 predicting species coexistence, we used the simulations of mutual invasion (simulations 3 and 4),  
999 described above, to construct pairs of each species' frequency and their growth rate in a saturated  
1000 community. For all of these simulations, we used only supply concentrations of the resources  
1001 that are known to allow for coexistence. For each value of a species frequency between 0 and 1,  
1002 we calculated the slope of growth rate versus frequency. Figure A1 shows that this slope is not  
1003 constant and actually changes sign depending on the species' frequencies used. Thus, using only  
1004 the slope of the NFD relationship is inadequate to predict coexistence.

1005           Next, we used both the slope and elevation from the NFD method to extrapolate to  
1006 frequency of 0 and predict whether the species is capable of invasion from rare (Figure A2  
1007 panels A and B). Figure A2 shows that for supply conditions known to allow coexistence, the  
1008 accuracy of the predictions from the NFD method depends on the range of frequencies over  
1009 which the slope of NFD was measured. We discuss two points (A and B in Figure A2) to explain  
1010 this effect. At the point Labeled “A”, the slope of NFD for species 1 predicts a positive growth  
1011 rate as frequency approaches 0, but at the complementary frequency of species 2, the slope of  
1012 species 2’s NFD predicts a negative growth rate when rare. However, based on the other 3  
1013 methods, numerical simulation, and Tilman’s resource ratio theory, the species are predicted to  
1014 coexist. Thus, measuring NFD under the red regions in Figure A2 will incorrectly predict  
1015 exclusion even though the species will coexist. At the point labeled “B” in Figure A2, the slope  
1016 and elevation of NFD for both species predicts a positive growth rate when rare. This region,  
1017 depicted in blue, includes the equilibrium frequency for the two species. If an empiricist made  
1018 their measurements between frequency of ~0.05 to ~0.85 for species 1, and used the slope of  
1019 NFD, they would incorrectly predict that the species will not coexist. Since the frequency at  
1020 which the species reach equilibrium depends on the resource supply ratio, there is no single  
1021 frequency of the species that consistently leads to the correct predictions (Figure A2). While  
1022 certain intermediate frequencies of the two species can be used to make accurate predictions, an  
1023 empiricist would not know these frequencies without performing the competition experiments or  
1024 examining frequency dependence across the entire range of frequencies. As a result, the only  
1025 reliable way of implementing the NFD is to measure the slope and elevation for each species  
1026 where its frequency approaches zero.

1027           For the NFD method, accurate predictions required that the slope of NFD was evaluated  
1028    approaching frequency of zero for each species (i.e. invasion conditions). In Figure S2 d-f, we  
1029    show that evaluating the NFD slope at other frequencies leads to the wrong predictions. We used  
1030    the NFD plot to evaluate coexistence at three frequencies, including near 0% (panel e and h of  
1031    Figure S2), 50% (panel f and i of Figure C2) and near 100% (panel g and j of Figure C2), and  
1032    either with (panel e-g of Figure C2) or without considering the elevation in addition to the slope  
1033    (panel h-j of Figure C2). We see that using the NFD slope evaluated at near 0% frequency will  
1034    consistently yield accurate predictions of species coexistence that match the those of the other  
1035    methods.  
1036



1037

1038 Supporting Information Figure A1. Results of simulation experiments using the NFD method.

1039 Panels a and b show per capita growth rate versus the frequency of species 1 and 2. At any

1040 frequency of the two species, the NFD method requires that we use the slope to extrapolate and

1041 estimate the growth rate when frequency approaches zero (the extrapolated vertical intercept).

1042 For frequencies where this method predicts mutual invasibility for both species, i.e. species can

1043 coexist, the lines are blue. For frequencies of the two species where the method leads to the

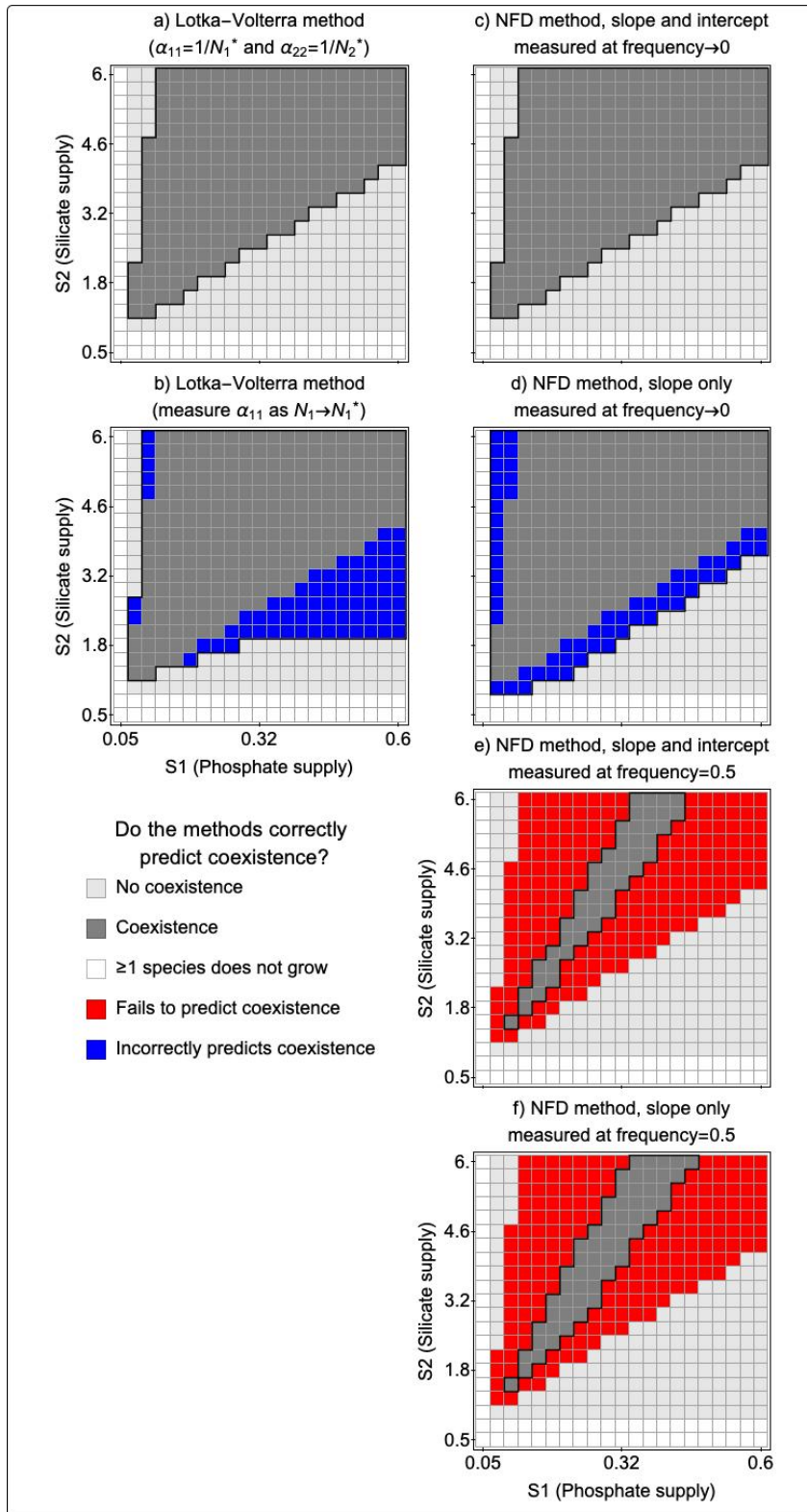
1044 incorrect prediction, the lines are red. Both species have positive growth rates when their

1045 frequency approaches zero, indicating that they are mutually invisable. Panel c shows the

1046 accuracy of the NFD method as a function of the supply Si:P ratio and the frequency of the two

1047 species at which the method was applied. The vertical dashed line represents the slice depicted in

1048 panels a and b. For all of the Si:P ratios shown in panel c, the species are mutually invisable and  
1049 will coexist. This plot indicates that using NFD will often predict that the species will not  
1050 coexist, when in fact they do coexist. This is important because without examining the full range  
1051 of species frequencies in an experiment, one would not know whether and where the relationship  
1052 between frequency and growth rate is non-linear.  
1053



1054

1055 Supporting Information Figure A2. Results of simulation experiments comparing predictions

1056 from the Lotka-Volterra and NFD methods.



1057 **Supporting Information B: Relating the Sensitivity Method to Chesson's Definition of ND**  
 1058 **and RFD Using the Lotka Volterra Model**

1059 Here we show that sensitivity method is identical to the Lotka Volterra method given the  
 1060 specific limiting assumptions of the sensitivity method. To do this, we derive the sensitivity  
 1061 metric ( $S_i$ ) from the Lotka-Volterra competition model (Equation 2). The  $\mu_i$  in Equation 5 is the  
 1062 maximum per capita growth rate in monoculture, equal to  $r_i$  in Equation 2. The  $\mu_{ij}$  is the invasion  
 1063 growth rate, so that we can replace  $N_j$  with species  $j$ 's carrying capacity,  $K_j$ , and replace  $N_i$  with  
 1064 0, so that  $\mu_{ij}=r_i(1-\alpha_{ij}K_j)$ . Using this substitution, we show in Equation B1 that the sensitivity  
 1065 metric ( $S_i$ ) is the equilibrium density of species  $j$  ( $K_j$ ) multiplied by the *per capita* competition  
 1066 coefficient ( $\alpha_{ij}$ ).

$$1067 \quad S_i \equiv \frac{\mu_i - \mu_{ij}}{\mu_i} = \frac{r_i - r_i(1 - \alpha_{ij}N_j^*)}{r_i} = \alpha_{ij}K_j \quad (\text{B1})$$

1068 Since the intraspecific competition coefficients in the Lotka Volterra model are equal to  
 1069 the inverse of the equilibrium population density for the monoculture ( $\alpha_{ii} = \frac{1}{K_i}$ ), the sensitivity  
 1070 metric can be shown to be equivalent to the ratio of interspecific to intraspecific interaction  
 1071 coefficients (Equation B2).

$$1072 \quad S_i = \alpha_{ij}K_j = \alpha_{ij} \frac{1}{\alpha_{ii}} = \frac{\alpha_{ij}}{\alpha_{jj}} \quad (\text{B2})$$

1073 From this substitution, we can relate the sensitivity metric to Chesson's ND (Equation B3), RFD  
 1074 (Equation B4), and use these estimates to assess the conditions for coexistence (Equation 1)

$$1075 \quad \text{ND} = 1 - \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}} = 1 - \sqrt{S_i S_j} \quad (\text{B3})$$

$$1076 \quad \text{RFD} = \sqrt{\frac{\alpha_{ij}\alpha_{ii}}{\alpha_{jj}\alpha_{ji}}} = \sqrt{\frac{S_i}{S_j}} \quad (\text{B4})$$

1077 **Supporting Information C: Relating the Negative Frequency Dependence Method to**  
1078 **Chesson's ND and RFD**

1079 Here we show that in order for the NFD slope to be constant, the community density  
1080 must be both saturated and fixed across all frequencies of the species. To do so, we attempt to  
1081 derive the NFD slope and intercept from the two species Lotka-Volterra competition model  
1082 (Equation 1). Since there is no variable representing each species' frequency in the Lotka-  
1083 Volterra model, we have to assume a fixed community density,  $B$ . This assumption also satisfies  
1084 the assumption of the NFD method that the community density is always saturated. Fixing the  
1085 community density makes the interspecific density dependence,  $\alpha_{ij}$ , equivalent to frequency  
1086 dependence (Adler et al. 2007), and allows species' frequency to be represented by  $N_i/B$ . The  
1087 two-species Lotka-Volterra competition model can then be rewritten as follows

1088 
$$\frac{1}{N_i} \frac{dN_i}{dt} = r_i \left( 1 - \frac{\alpha_{ii}N_i + \alpha_{ij}(B - N_i)}{B} \right) \quad (C1)$$

1089 , where  $B$  is the fixed community density and one unit decrease of  $N_i$  will lead to one unit  
1090 increase of  $N_j$ . From Equation C1, we derive the NFD slope and intercept in the following  
1091 equations.

1092 
$$NFD \text{ slope} \equiv \frac{\partial \frac{1}{N_i} \frac{dN_i}{dt}}{\partial \frac{N_i}{B}} = \frac{\partial \frac{1}{N_i} \frac{dN_i}{dt}}{\frac{1}{B} \partial N_i} = B \frac{\partial \frac{1}{N_i} \frac{dN_i}{dt}}{\partial N_i} = r_i (\alpha_{ij} - \alpha_{ii}) \quad (C2)$$

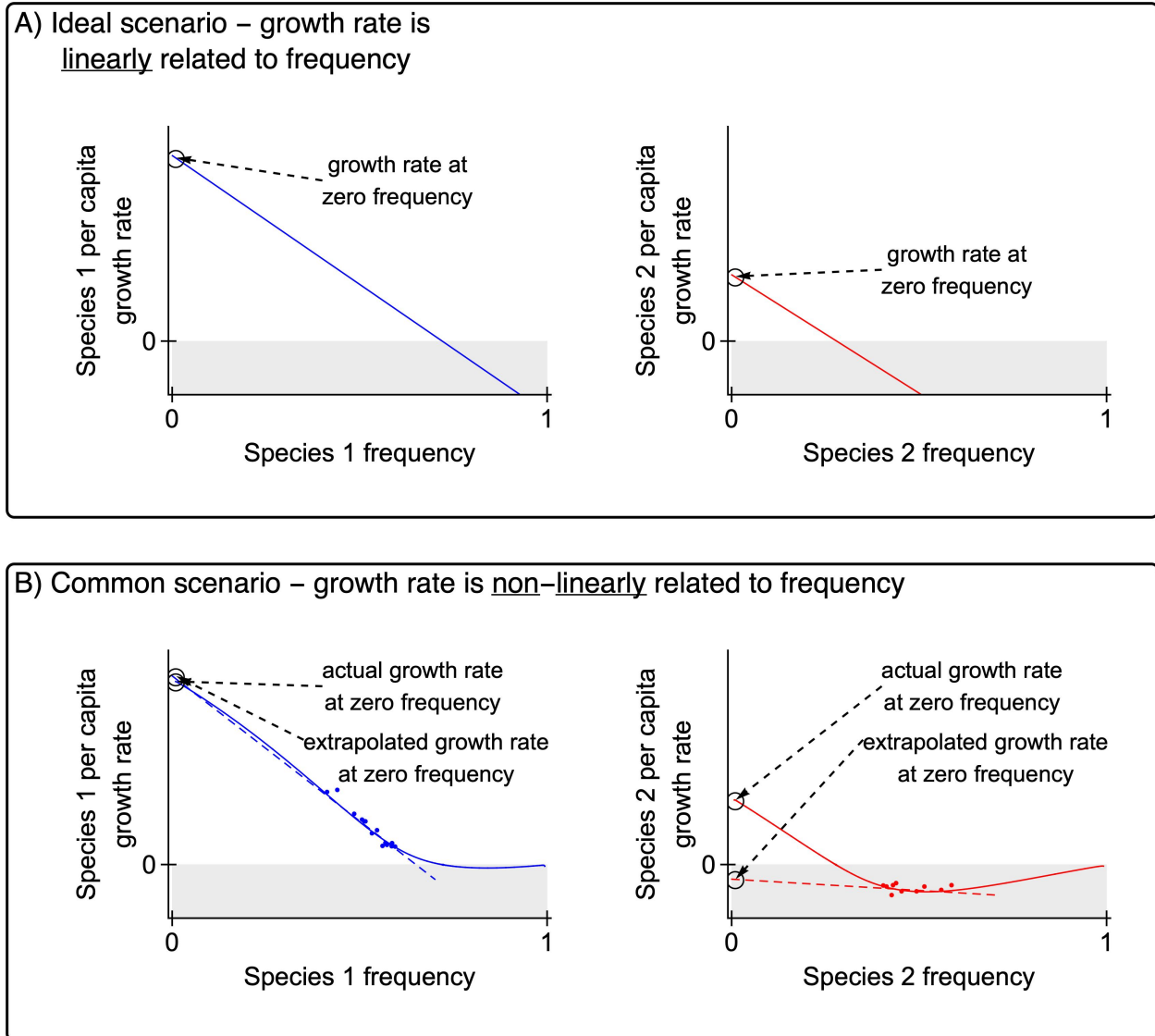
1093 
$$NFD \text{ intercept} \equiv r_i (1 - \alpha_{ij}) \quad (C3)$$

1094 In Equation C2 the NFD slope becomes constant, which means that species' per capita growth  
1095 rate linearly depends on its frequency.

1096 In addition, from Equations C2 and C3, we argue that both NFD intercept and slope  
1097 should be used with caution in evaluating Chesson's inequality. First, the NFD intercept  
1098 represents whether species can successfully invade a steady-state population of its competitor at

1099 its carrying capacity, so it can be used to accurately assess mutual invasibility. However, neither  
1100 the difference nor the ratio of two species' NFD intercepts (Equations C2 and C3) take an  
1101 analogous form to Chesson's definition of ND and RFD. It is worth noting, however, that the  
1102 slope of NFD relationship has been used to represent ND for annual plant communities (Yenni et  
1103 al. 2012, Yenni et al. 2017). Thus, while the NFD method can correctly predict mutual  
1104 invasibility, the NFD intercept and slope should not be interpreted as RFD and ND in order to  
1105 evaluate Chesson's inequality.

1106         The utility of the NFD method depends on its ability to correctly predict whether species  
1107 have positive growth rates when their frequencies approach zero. If the relationship between a  
1108 species frequency and its growth rate is non-linear, however, then the accuracy of the NFD  
1109 method is critically dependent on the range of species frequencies used by an empiricist. In  
1110 Figure C1 we show that the NFD relationship is non-linear even when the underlying population  
1111 dynamics are governed by the Lotka-Volterra model. The result of this non-linearity is that,  
1112 depending on the range of species' frequencies used to estimate the NFD slope and intercept, this  
1113 method can give inaccurate predictions.



1114

1115 Figure C1: Panel A shows a hypothetical situation where species frequencies are linearly related  
1116 to their growth rate and an empiricist can extrapolate to predict growth rates at frequency of zero  
1117 and diagnose mutual invasibility. Panel B shows the more likely scenario in which growth rates  
1118 are non-linearly dependent upon species frequencies. These plots were made using numerical  
1119 simulation of the two-species Lotka Volterra Model, using parameter values that should allow  
1120 for coexistence (at frequency of 0.72 for species 1). The points in Panel B represent empirical  
1121 measurements collected at intermediate frequency of both species. Using those measurements

1122 and extrapolating to zero frequency yields the incorrect prediction that the species will not  
1123 coexist.